IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

PLANT PHYSIOLOGY

EDITORS

CHARLES A. SHULL

CHARLES B. LIPMAN
BURTON E. LIVINGSTON
CARLETON R. BALL
FRANCIS E. LLOYD

VOLUME 9

1934

WITH THREE PLATES AND ONE HUNDRED AND EIGHTY-NINE FIGURES

SCIENCE PRESS PRINTING CO.
LANCASTER, PA.

TABLE OF CONTENTS

NUMBER 1. JANUARY

PAGE

Relations of germinating soy beans to temperature and length of incubation time ..... T. I. EDWARDS 1

Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple ..... VICTOR A. TIEDJENS 31

Chlorophyll production under various evironmental conditions. GUS B. ULVIN 59

Responses of Kentucky bluegrass to variations in temperature, light, cutting, and fertilizing ..... CARTER M. HARRISON 83

Hydration of solute ions in relation to acidity, alkalinity, and pH. LEWIS H. FLINT 107

Influence of shorter light rays upon assimilation of nitrate by the young wheat plant. W. E. TOTTINGHAM, H. L. STEPHENS, and E. J. LEASE 127

Amounts of bound and free water in an organic colloid at different degrees of hydration ..... HELEN L. CHRYSLER 143

Gradient composition of rose shoots from tip to base. H. B. TUKEY and E. L. GREEN 157

Significance of transpiration ..... HARRY F. CLEMENTS 165

Effect of soft x-rays on germination of wheat seeds. H. M. BENEDICT and H. KERSTEN 173

Absorption spectra of alpha and beta carotenes, and leaf xanthophyll at room and liquid nitrogen temperatures ..... ELMER S. MILLER 179

Soluble solids in the watermelon ..... LOWELL R. TUCKER 181

Notes 183

NUMBER 2. APRIL

Nutritional requirements of the root rot fungus, *Phymatotrichum omnivorum.* WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE 187

Effects of humidity on metabolism in tomato and apple. G. T. NIGHTINGALE and J. W. MITCHELL 217

The soil as direct source of carbon dioxide for ordinary plants. BURTON E. LIVINGSTON and RUTH BEALL 237

Causes of blind wood in roses ..... DONALD S. HUBBELL 261

Relation between root respiration and absorption ..... LETA HENDERSON 283

Carbohydrate-nitrogen and base element relationships of peas grown in water culture under various light exposures ..... ORMAN E. STREET 301

PAGE

Application of calorimetric methods to ecological research.
FRANCES L. LONG 323
Carbon dioxide content of the gas from pea pods ..... Z. I. KERTESZ 339
Metabolism of etiolated seedlings as affected by ammonium nutrition.
LELAND BURKHART 351
Production of alcohol and acetaldehyde by tomatoes.
FELIX G. GUSTAFSON 359
Comparison of the heating and freezing methods of killing plant material for cryoscopic determinations ..... T. D. MALLERY 369
Influence of thyroxin on the growth of plants ..... ELIZABETH ELLEN DAVIS 377
Effect of high frequency sound waves on oxidase activity.
RALPH J. CHRISTENSEN and RUDOLF SAMISCH 385
Lateral water transfer in leaves of *Ginkgo biloba* ..... CHARLES A. SHULL 387
Comparison of anatomical and histological differences between roots of barley grown in aerated and in non-aerated culture solutions.
A. E. BRYANT 389
Tolerance of liquid-air temperatures by seeds of higher plants for sixty days ..... C. B. LIPMAN and G. N. LEWIS 392
Notes 395

NUMBER 3. JULY

Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents.
CONSTANCE ENDICOTT HARTT 453
Some effects of potassium upon the amounts of protein and amino forms of nitrogen, sugars, and enzyme activity of sugar cane.
CONSTANCE ENDICOTT HARTT 452
Germination of the red spruce ..... HENRY IVES BALDWIN 491
Carbohydrate and nitrogen relations in wheat plants with reference to type of growth under different environmental conditions.
ANNIE M. HURD-KARRER and ALLAN D. DICKSON 533
Physiological aspects of the effect of continuous soil aeration on plant growth ..... W. F. LOEHWING 567
Potentially unlimited growth of excised tomato root tips in a liquid medium ..... PHILIP R. WHITE 585
Salt concentration and reversibility of ice-formation as related to the hardiness of winter wheat ..... S. T. DEXTER 601
Effect of soil temperature on transpiration in *Helianthus annuus*.
F. E. CLEMENTS and E. V. MARTIN 619
Relation of benzoic acid content and other constituents of cranberries to keeping quality ..... J. A. CLAGUE and C. R. FELLERS 631
Effect of ethylene, ethylene chlorohydrin, and ultra-violet light on carbohydrate content of stored apples ..... ROBERT B. DUSTMAN 637

PAGE

Long and short wave-length limits of photosynthesis.
G. RICHARD BURNS 645

Evaporation in its thermodynamic relation to the cohesion theory and to imbibitional and osmotic pressures of cell constituents.
CLYDE HOMAN, T. F. YOUNG, and CHARLES A. SHULL 653

Preparation of humate iron and other humate metals.
C. KENNETH HORNER, DEAN BURK, and SAM R. HOOVER 663

Chemical changes in carrots during growth HANS PLATENIUS 671

Application of quantitative spectral analyses to binary mixtures of the common carotenoids ELMER S. MILLER 681

What the organic chemist can do for plant physiology.
HUBERT BRADFORD VICKERY 685

A large-capacity drying oven with constant uniform temperature and forced ventilation EMMETT V. MARTIN 689

Quantitative absorption spectra of the common carotenoids.
ELMER S. MILLER 693

Notes 695

NUMBER 4. OCTOBER

Penetration and accumulation of petroleum spray oils in the leaves, twigs, and fruit of citrus trees P. W. ROHRBAUGH 699

Carbohydrate-nitrogen ratios with respect to the sexual expression of hemp PAUL J. TALLEY 731

Losses to the corn crop caused by leaf injury GEORGE H. DUNGAN 749

Factors in elongation and expansion under reduced light intensity.
FREDERIC E. CLEMENTS and FRANCES L. LONG 767

The cohesion theory of transpiration ROBERT E. HUNGATE 783

Freezing phenomena in cresoap emulsions of petroleum oils.
PAUL A. YOUNG 795

Production of vitamins by a pure culture of *Chlorococcum* grown in darkness on a synthetic medium.
M. F. GUNDERSON and C. E. SKINNER 807

Preparation of aqueous extracts of soluble nitrogen from plant tissues.
O. W. DAVIDSON, H. E. CLARK, and J. W. SHIVE 817

Relation of certain amino acids to carbon dioxide and mycelium production of *Fusarium oxysporum*.
ARTHUR K. ANDERSON and KATHRYN EMMART 823

Respiratory rate and enzyme activity as related to the hardened condition of plants S. T. DEXTER 831

Method for the preparation of green plant material for the extraction of juices L. D. DONEEN 839

Nuclear division of *Tradescantia virginiana* L.
BEATRICE LEHMAN and F. M. ANDREWS 845

PAGE

GOTTLIEB HABERLANDT — A. C. Noé — 851

Demonstration of some of the mechanisms involved in the ascent of sap in plants — ERNEST H. RUNYON — 856

A note on fruiting apple spur phyllotaxy — HAROLD L. COLBY — 858

Notes — 861

ERRATA

VOLUME 9

Page 61, line 8 from bottom, dele period and word "then," and place remainder of sentence in parentheses.

Page 76, table XIV, bottom of column 4, for "0.372" read 0.0372.

Page 78, line 16, for "6.67" read 5.56.

Page 100, fig. 7 replaced by cut provided with April number.

Page 115, lines 12 and 13, transpose $Cl^{(-)h} = 158$ and $K^{(+)h} = 94$.

Page 128, line 12, for "114" read 14.

Page 179, table I, transpose position of "Alpha carotene" and "Beta carotene" in first column.

First cover page, April number, line 15 table of contents, for "SWEET" read STREET.

First cover page, July number, line 8 table of contents, for "452" read 453.

Fourth cover page, July number, lines 7 and 8, for "University of Michigan" read Cornell University.

Vol. 9 No. 1

PLANT PHYSIOLOGY

JANUARY, 1934

RELATIONS OF GERMINATING SOY BEANS TO TEMPERATURE AND LENGTH OF INCUBATION TIME[1]

T. I. EDWARDS

(WITH SIX FIGURES)

Introduction

Because much physiological study deals with plants that are secured from seeds, the conditions that prevail during the preliminary germination period constitute a very important part of such studies. Individual variability among the similar plants of an experimental series makes physiological experimentation very laborious, and presents one of the most difficult problems with respect to both technique and interpretation. Many recent experimenters have attempted to reduce variability through some sort of plant selection, and such selection usually begins with the seeds employed, —as when the seeds are chosen according to their genetic background and with reference to size, weight, color, etc. Young seedlings, just after germination, are commonly subjected to more or less rigorous selection according to size and general appearance, but especially according to apparent vigor. Such selection tends to restrict variability and furnishes experimental series of plants that are more nearly alike in their intrinsic physiological nature, as well as in their variability, than would be the case without selection. When young seedlings are selected for uniformity, the standard set of conditions that prevailed while germination was in progress becomes a basis for the selection. Consequently any advance in our knowledge of how specified lots of seed may be influenced in their germination performance by the environmental complex prevailing during the period of germination should be useful in the preparation of seedlings for experiments dealing with more advanced developmental phases. In this connection see GOODSPEED (11), CORRENS (5), HAASIS (12), and TANG (36). Furthermore, a number of students (20) have presented evidence leading to the

[1] Botanical contribution from the Johns Hopkins University, no. 118.

supposition that the environmental conditions prevailing during seed germination may exert a persistent influence on the vigor and capacity for response of the resulting plant; in other words, conditions that were effective during seed germination may limit subsequent growth and yield. From these and other considerations it is evident that the subject of seed germination is an important one, not only in itself but also as fundamental to the study of more mature plants.

From the standpoint of experimental technique, studies on seed germination are in many ways much less difficult than studies dealing with more mature phases of growth and development. In many instances germination may be studied in the absence of illumination, with the avoidance of many difficult and complicated considerations that inevitably present themselves when cultures are carried out in light; for light intensity, light quality, and light fluctuation may all be left out of consideration in experiments on germination of many common sorts of seed, since ordinary seeds generally carry sufficient nutrient material to support germination and the early stages of seedling development. Although it is desirable to employ as germination medium a weak solution of inorganic salts rather than distilled water, yet excellent germination may be secured with a great variety of different solutions (**10**).

The experiments with which this paper deals show, for a specified lot of Black Eyebrow soy bean seeds, the influence of maintained temperature on the proportion of seeds germinating with incubation periods of different lengths. For a number of different combinations of temperature and time the resulting percentage values were studied by simple statistical methods, to bring out some characteristics of the variability of the lot of seed used. For each maintained temperature tested, increments of germination percentage for successive time intervals were studied.

Maintained temperature and time were the only experimental variables involved. All other influential conditions were alike for all tests, or else their complex differed from test to test only as they may have been influenced by temperature and time. Of the various influential conditions that may be varied in an experimental study, maintained temperature is especially suited to experimentation and analysis because it acts directly upon the seed protoplasm and is not subject to modification by integuments or protoplasmic membranes. For these tests temperature was an independent variable, depending only on the environment and not being significantly influenced either by the nature of the seeds or by their activity. The duration factor, time, is naturally an unavoidable component of any experimental procedure, and the length of exposure to a given set of conditions has a powerful influence on the organism's responses.

Soy bean (*Soja max* Piper or *Glycine hispida* (Moench) Maximowicz) seed was chosen as the subject of this study for several reasons. Soy bean is an important crop plant in many regions, and it has been used in a number of physiological studies as well as in field experimentation. The seeds are of convenient size for such experiments, and they germinate promptly in darkness when other conditions are suitable. A commonly cultivated, natural hybridization between different varieties of this species appears to be uncommon (**8**) and homozygous lots of seed are consequently obtainable.

A general review of the literature on temperature relations and time relations of germinating seeds is already available (**6**), and only the publications that bear directly on this study will be mentioned here. Several studies on the germination of soy bean seeds and the early growth of seedlings have been reported recently. WILSON (**40**) and OATHOUT (**28**) studied the general temperature relations of germinating soy bean seed. Employing microchemical methods, VON OHLEN (**38**) traced the major metabolic changes of early stages of development of Manchu soy beans. HAFENRICHTER (**15**) and STARK (**35**), using the same stock of seed, investigated CO_2 output and change in amino-acid content in seedlings maintained at different temperatures. JONES and TISDALE (**19**) cultured soy bean plants in pots of soil maintained at different temperatures between 12° and 40°, while the shoots were all exposed to approximately similar greenhouse conditions, the air temperature being 14°–18° for all cultures. These plants tolerated soil temperatures between 12° and 36° but failed to grow when the temperature of the soil was 40°. Root development was about the same for all soil temperatures tested above 18°. The greatest dry weight of tops was secured with soil temperatures between about 24° and about 35°. Nodule development, reckoned on the basis of dry weight, was most vigorous at a soil temperature of 24°. On the basis of JONES and TISDALE'S tests, the soy bean plant appears to be well adapted to a range of soil temperatures much broader than the range tolerated by many other crop plants that have received attention in this respect.

Experimental methods and primary numerical results

With the exception of the maintained temperatures, a uniform procedure was followed for all the experiments. The seeds lay on agar plates in covered cylindrical petri dishes 10 cm. in diameter and 1.5 cm. deep. Each experiment embraced four sets of five dishes, and each dish had 20 seeds.

The battery of maintained-temperature chambers of this laboratory (**23**), which has served for many earlier studies, was employed, but only five of the seven chambers were actually used. The automatic controls

were set so as to provide the following five temperatures: 24.5°, 28.5°, 33.0°, 36.5°, and 40.0°. The two unused chambers had temperatures of 20° and 17°. The range of temperature fluctuation for any of the used chambers was about ± 0.5°.

In this apparatus the seven chambers are in a linear series, each provided with a continuously stirred water-jacket. Each water mass is separated from the two adjacent ones by means of an uninsulated sheet-iron partition, and the entire series is inclosed in a light-proof cabinet with hair-insulated walls and bottom. Access to the chambers is had from above, by means of removable lids with cork and wood insulation. Heat is supplied at one end of the series from a thermostatically controlled tank of hot water and it is removed at the other end by means of another tank of water, the latter being thermostatically controlled by means of a mechanical refrigeration machine. The chambers are vertical cylinders (38 cm. in diameter and 38 cm. deep), and all parts of each chamber remain at practically the same temperature, but the temperature of each chamber differs from the temperature of adjacent chambers to a degree determined by the settings of the two thermostats. In each chamber the petri-dish cultures were stacked loosely, in horizontal position, in a suspended basket of perforated sheet metal.

The seeds used were all from a single lot of Black Eyebrow soy bean seed grown in Indiana in 1930, supplied by the Eastern States Farmers Exchange through the courtesy of Mr. C. W. Clemmer, of Springfield, Mass. It was received in December, 1930. These seeds were characterized by a black hilum and by a black area on each side of the hilum, the rest of the seed surface being olive green or brown. The seed-coat markings corresponded to the description given by MORSE (**26**) for seeds of this variety, and the seeds of this lot were very similar to those of other lots of the same variety obtained from other sources, although those of the lot used were a little smaller on the average; 100 of them weighed 14.1 gm. There was considerable variation in the relative sizes of the areas occupied by the two colors but no obvious relations were detected between seed-coat markings and germination behavior. The whole stock of seeds was sorted at the beginning of the study and a small proportion of obviously imperfect or notably unusual individuals was discarded. The remaining supply was stored in a cloth bag in a loosely covered metal can kept in a basement room. OATHOUT (**28**) found such treatment very satisfactory in preserving the vitality of his soy bean seed. The experiments were performed between February 6 and March 22, 1931, so that none of the seeds used differed in age by more than a month and a half.

The medium for these tests was an agar gel containing per liter: 5.0 gm. of the Digestive Ferment Company's "Bacto-agar," 0.0018 mol. (0.2451

gm.) of KH_2PO_4, 0.00052 mol. (0.1228 gm.) of $Ca(NO_3)_2 \cdot 4H_2O$, and 0.0015 mol. (0.3707 gm.) of $MgSO_4 \cdot 7H_2O$. The salts were used because a dilute solution had been found by many workers to be more satisfactory than distilled water as a medium for seed germination. The solutes used in this way have in many instances been considered as "seed stimulants," and a great number of organic as well as inorganic substances have been assigned to this category by various investigators; NIETHAMMER (**27**) has published an extensive summary concerning seed stimulation. When seeds are germinated (and even when just preliminarily soaked) in distilled water, it is always possible that substances emanating from the seeds or from accompanying microorganisms may so modify the adjacent medium as to affect germination, and it appears that such effects are not so apt to occur when a dilute salt solution is used instead of water. Loss of essential ions from the swelling and germinating seeds appears to be retarded by the presence in the medium of suitable salts at suitable concentrations. The small traces of injurious substances that are apt to be present in distilled water seem generally to be robbed of their toxicity when nutrient salts are also present at low but considerable concentrations. GERICKE'S (**10**) experiments on germinating wheat seed showed no consistent differences in the physiological effects of dilute 3-salt solutions having very different partial concentrations of the salts. His solutions were all markedly better than distilled water.

The three salts were first dissolved in distilled water from a Barnstead still, to make SHIVE'S (**33**) solution R5C2 (0.175 atm.). (This low total concentration is the same as was used by GERICKE, being only one-tenth as great as the lowest total concentration used in SHIVE'S series of experiments.) To 2.5 liters of the 3-salt solution thus prepared (the pH value of which was found to be 4.3), 12.5 gm. of "Bacto-agar" were added and the mixture heated in a beaker over a boiling water-bath until all granules had disappeared. Then distilled water was added to make the volume up to the original one, 2.5 liters. Each stock of gel thus prepared was stored in stoppered flasks at 2°–3°. Storage was never for more than five days, in which time no apparent alteration of the gel occurred. Its pH value was 5.6, as ascertained by the use of a quinhydrone electrode as well as by colorimetric means.

A few hours before a series of cultures was to be started, a flask of gel was removed from the refrigerator and heated in a water-bath until liquified. Then 25 ml. were pipetted into each of 20 heat-sterilized petri dishes, which were immediately placed on a previously cooled slate slab, where the agar plates rapidly solidified. This rapid cooling saved some time and lessened the amount of water that condensed on the lids of the dishes during the cooling process.

Twenty seeds, taken at random from the stock, were about equally spaced on each of the 20 plates, each seed lying on its side. The seeds sank slightly into the agar gel, and at the end of 12 hours about one-third of the seed volume was below the gel surface, the hilum being just above the surface. Thus a large area of the testa was in contact with the gel, which favored water absorption while gaseous exchange between seed and atmosphere was not greatly retarded. Rapid absorption of water took place, showing that the seed testas were readily permeable. About 25 minutes was required to distribute 400 soy beans on 20 agar plates.

It will be noted that no preliminary sterilization treatment was applied. While such treatments undoubtedly may be effective to prevent or retard subsequent development of microorganisms in the cultures, yet they are apt to exert some influence on germination. As has been remarked, a great variety of chemical substances have been observed to act as seed stimulants, and consequently it seems unlikely that any soaking treatment drastic enough to kill microbial spores would leave the seed embryo unaltered, aside from the effect of water absorption during the treatment (**9**). Preliminary soaking, which usually accompanies sterilization treatment, may in itself exert some influence on subsequent germination performance (**20**). It was found, for example, that Manchu soy beans that had received a preliminary soaking of one hour in water or in dilute solutions of ethyl alcohol required less time for germination on agar plates than was required when similar seeds had been placed on the plates without preliminary soaking. Many of these seeds were obviously injured in a single hour of soaking, however; in many instances the cotyledons were cracked or broken loose from the embryo or the testa was torn. Also the soaked seeds showed greater variability in the time required for germination than was shown by seeds laid on the agar dry. Since preliminary soaking generally hastens subsequent germination, it might be employed in studies on seed of Black Eyebrow or other varieties of soy bean if suitable time and temperature conditions were established, and if a suitable standard solution were used.

Immediately after placing the seeds, the 20 cultures of a series were installed in the requisite temperature chambers where they remained from 24 to 28 hours, except that they were removed for observation at 2-hour intervals after germinated seeds began to appear. Preliminary experiments showed when germination was to be expected at the several temperatures, and so the cultures were usually not removed for examination before the end of the tenth or twelfth hour of incubation. A culture was never out of its chamber for more than four or five minutes at any observation. The cultures were held above the level of the eye and viewed from beneath at each observation; the white radicles showed up distinctly and it was easy to decide whether a seed had germinated or not. All germinated seeds

were removed as they were found and their number was recorded. The seeds were never touched with the fingers, either in the original sorting, in placing them on the plates, or in removing or transferring them; bone-tipped forceps were always employed. The tests were discontinued before all the seeds that might have germinated had done so, because the 2-hour increments of germination percentage became small and highly variable with longer incubation periods. Satisfactory data on these later increments might have been secured only with a much larger number of repetitions than was needed to give significant values for the earlier ones.

These seeds were only rarely observed to be attacked by molds; even at the highest temperature tested such contamination was slow to appear. Whenever an ungerminated seed was seen to be attacked by mold to such an extent as was judged to threaten the contamination of neighboring seeds it was removed. Sometimes such moldy seeds were discarded but in many instances they were simply transferred to vacant depressions in the same agar plate, left by the previous removal of germinated seeds. Usually these transplanted seeds proceeded to germinate without noticeable retardation.

For this study a seed was considered to have germinated when the radicle had just broken the testa. The radicle usually grew downward within the seed coat and emerged on the lower side.

For recording germination percentages, the experimental unit was usually a set of five agar plates bearing altogether 100 seeds, 20 on each plate. The total number of seeds that had germinated in any set before any observation was consequently the germination percentage for that set and for the corresponding temperature and length of incubation period. Each test comprised four of these 100-seed sets, all incubated simultaneously at the same maintained temperature. Consequently there were four primary percentage values for each period of incubation in each test. Every test was repeated until it appeared that the range of variation of the last four primary percentage values for each length of incubation period was well within the range of variation of all corresponding values previously recorded.

There were finally available a large number of comparable primary percentages for each combination of temperature and time. The arithmetical mean and the standard error of the mean were computed for each combination, and these values appear in table I, separated by the usual $\pm$ sign. In this table the number of primary percentage values on which each mean is based is shown in parentheses below the mean. Combinations that showed no germination are indicated by dashes. The formula used by COLLINS (4) was employed in computing the standard error, namely,

$$\sigma_M = \sqrt{\frac{\sigma^2}{N-1}}$$

in which σ_M is the standard deviation of the mean (or the standard error), σ represents the standard deviation of individual values, and N is the number of individual values available,—in this case the number of primary percentage values for the temperature-time in question.

TABLE I

GERMINATION PERCENTAGES OF BLACK EYEBROW SOY BEANS WITH DIFFERENT COMBINATIONS OF TEMPERATURE AND INCUBATION TIME

INCUBATION TIME	24.5°	28.5°	33.0°	36.5°	40.0°
hr.					
12	---	0	3.0 ± 0.4 (12)	1.3 ± 0.4 (12)	---
14	---	3.4 ± 0.2 (8)	16.8 ± 0.6 (20)	13.5 ± 0.9 (20)	---
16	---	17.4 ± 2.2 (16)	39.9 ± 0.5 (24)	36.4 ± 1.2 (24)	---
18	6.5 ± 0.3 (12)	38.0 ± 2.0 (16)	55.6 ± 0.9 (20)	56.5 ± 1.2 (37)	3.8 (2)
20	21.6 ± 2.1 (12)	52.6 ± 1.7 (12)	70.3 ± 1.3 (12)	69.4 ± 0.8 (28)	15.0 ± 0.1 (10)
22	37.4 ± 2.2 (12)	67.8 ± 1.9 (11)	76.8 ± 1.5 (8)	76.3 ± 1.2 (21)	39.3 ± 2.2 (10)
24	51.6 ± 2.2 (12)	77.0 ± 1.6 (7)	80.8 ± 1.4 (8)	79.4 ± 1.2 (13)	58.6 ± 1.6 (10)
26	63.4 ± 0.9 (12)	---	---	84.4 ± 1.6 (12)	70.0 ± 1.6 (8)
28	70.6 (4)	---	---	---	72.4 (4)

As might be expected, there was considerable variation in each series of primary percentage values. The characteristics of the variation usually encountered are shown by the following representative frequency tabulation for the combination of 18 hours and 36.5°. (In the preparation of this tabulation the experimental unit was a single agar plate, instead of five plates, and there were 183 comparable primary values. The largest possible value is of course 20 instead of 100.)

Seeds germinated:	5	6	7	8	9	10	11	12	13	14	15	16	17
Frequency:	0	5	9	8	19	26	34	26	22	21	10	3	0

For this series the average number of germinated seeds is 11.1 and the value for the crude mode is 11.0.

These data are presented graphically by the frequency polygon of figure 1, where the possible values for the number of germinated seeds per plate are plotted on the horizontal axis and the frequency with which each

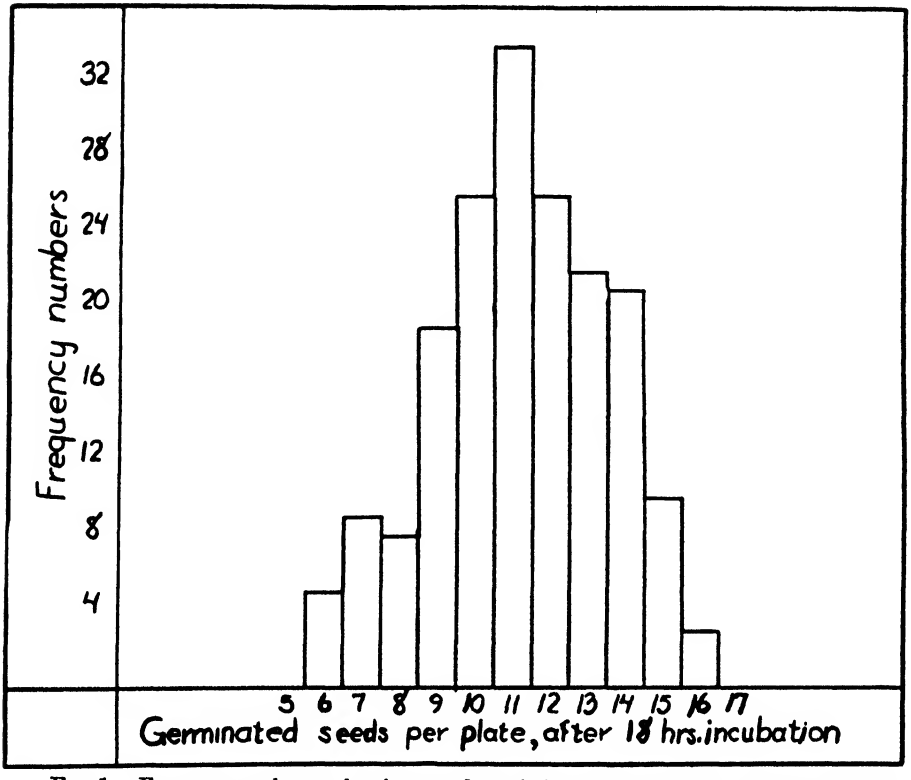


FIG. 1. Frequency polygon showing number of times that various numbers of Black Eyebrow soy bean seeds were found germinated after 18 hours of incubation at 36.5° (based on data from 183 plate cultures, each with 20 seeds).

value occurred is plotted as the height of a rectangle. This polygon indicates that a large proportion of the individual values falls close to the average value (11.1) and in approximately equal numbers on either side of it, and also that any percentage value occurs less frequently as its magnitude is farther removed from that of the average. In these general respects this polygon is similar to the curve representing the theoretical distribution of errors. These observations, and similar ones derived from tests with other temperature-time combinations, appear to justify the use of the averages, each with its standard error, as indices of the general performance of the lot of seeds with which this study deals.

Two sets of criteria might be used for judging the extent of the deviation of the individual values: (1) frequency polygons (such as that of figure 1) constructed from the values used in the computation of the means; and (2) the standard deviation of the means, which are statistical constants. The polygon of figure 1 may serve as a representative sample in detail, but

the tendency for the values of any comparable series to fall close to their arithmetical mean, and hence the relative significance of the latter in representing the whole series, is indicated by the standard error of the mean.

As has been mentioned, each culture was temporarily removed for a short time from its temperature chamber whenever an observation was made; *i.e.*, at 2-hour intervals after the lapse of 10 or 12 hours. All the petri dishes of a set were removed from the chamber together and were returned together after observation. The greenhouse room in which the temperature chambers are, and in which the cultures were examined, had an air temperature usually between 20° and 25° C., and each culture naturally tended to assume the room temperature, becoming either warmer or cooler, while it was out of its chamber. Also, while outside of their chambers the petri dishes and their contents were illuminated, although they were in darkness throughout most of the experimental period. It is conceivable that these short temporary interruptions in the otherwise even tenure of the maintained conditions of temperature and lack of illumination for any culture might exert some sensible influence on germination.

To determine whether this was really so, four special experiments were carried out with a temperature of 36.5°. In each of these experiments there were 25 dishes, 10 of which were removed, examined, and returned to the chamber in the usual way, at the end of 12, 14, 16, and 18 hours while the remaining 15 were not removed and examined until after 18 hours of incubation. For the 18-hour period the average germination percentage was 56.3 ± 1.20 for the first group (which had been subjected to temporary fluctuation) and the corresponding average for the second group was 54.5 ± 1.19. Since the difference between these values cannot be regarded as significant, it may be concluded that the environment changes in question did not alter the course of germination appreciably.

Discussion

General survey of results

Some of the general relations of the average germination percentages for the stock of Black Eyebrow soy bean seed used in the experiments are readily visualized when the averages of table I are plotted as graphs. A simple and direct graphic presentation of these data is shown in figure 2, which embraces seven separate temperature graphs, one for each of the different lengths of incubation period employed. Abscissae are maintained temperatures and ordinates are average germination percentages. Each graph represents the temperature relations of seedling production by this lot of seed in the specified time period. They appear like a group of slightly asymmetrical, downwardly concave curves, with a common maxi-

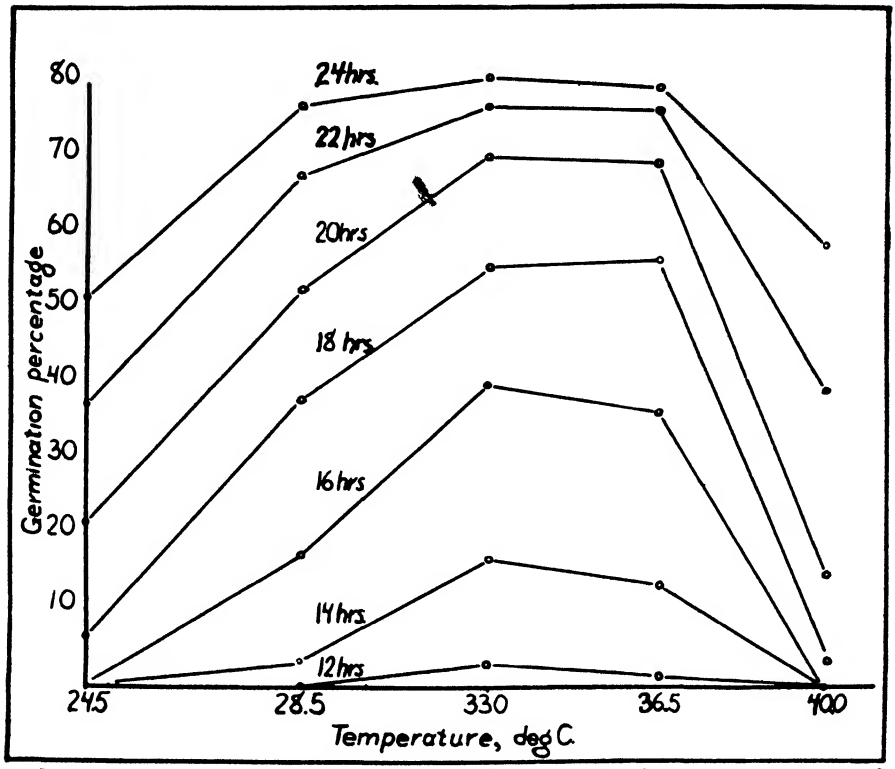


FIG. 2. Temperature-germination graphs for Black Eyebrow soy bean seed, for several lengths of incubation period. Each period length is represented by a separate graph.

mum in the region representing a temperature range from about 33.0° to about 36.5°. Each curve has a steeper slope on the high-temperature side than on the low-temperature side of its peak. For each different length of tested incubation period the temperatures 33.0° and 36.5° were in close agreement with respect to their average germination percentages, but of course the averages are greater as the period was longer. The three other temperatures all gave lower percentages. Cultures at 28.5° gave somewhat lower germination percentages than cultures of the same age at 33.0° or at 36.5°, but those at 28.5° always gave values higher than the corresponding ones at 24.5° or 40.0°. The optimal temperature range for each of the different period lengths consequently extends from about 33.0° to about 36.5°. No shifting of this optimal temperature range with time, such as has been described by several experimenters with other kinds of seed and with various experimental methods, is apparent here.

Successively longer incubation periods broadened the temperature range within which germination was observed. For the 12-hour, 14-hour, and

16-hour periods, minimal and maximal temperatures for the occurrence of germination are apparent, since in these instances two or more of the tested temperatures failed to give any germination. For the longer periods, however, the minimal and maximal temperature values clearly fall outside the temperature range embraced by the tests. With increasing length of period the graphs become progressively flattened at the top and the several tested temperatures tend to approach equal effectiveness with respect to seedling production. WILSON (40) found that approximately the same percentage of soy bean seeds eventually germinated at each of the temperatures he tested between 10° and 30°.

In connection with the present study, some experiments with seed of the Manchu, Minsoy, and Tokyo varieties of soy bean showed optimal temperature ranges with upper limits below that of the optimal range for the Black Eyebrow variety as here shown; in most of these additional instances 33.0° was more favorable for germination than either 36.5° or 28.5°.

In addition to the results thus far mentioned, further examination of the data of table I brings out a number of less obvious relations, some of which are considered in the following sections of this paper. It is to be borne in mind that these results and statistical relations apply strictly only to the experiments of this study, to the particular stock of Black Eyebrow seed used, and to the specified conditions and technique of this experimentation. Time factors are of course approximated only in terms of the 2-hour observation intervals, and all critical temperatures, as well as the lower and upper limits of critical temperature ranges, are naturally to be regarded as ranges of temperature rather than as definite temperatures. With more laborious and more time-consuming experimental procedure, and with still further repetitions of the tests, these approximations might have been given a higher degree of precision, of course; but the numerical results appear to be sufficiently precise to furnish some additions to our knowledge of the performance of samples of germinating seeds. The following discussion will be in terms of temperature ranges rather than in terms of exact temperatures, and each critical temperature mentioned may be considered as approximate to within a few degrees. The need for brevity of statement and for general clearness frequently limits the extent to which considerations concerning relative degrees of precision may be mentioned throughout reports on many biological studies. It is interesting to note that the inevitably approximate nature of critical temperatures for seed germination was clearly emphasized by HABERLANDT (13) as early as 1874, who wrote:

"Denn die Temperaturgrenzen bei welchen eine Schwächung und Verlangsamung des Keimungsprozesses eintritt, ist keiner scharf gezogene

Linie, sondern einem Streifen zu vergleichen, der unter Umständen eine Verschiebung der Grenzlinie für den Samen einer bestimmten Qualität wohl gestattet. Die mehr oder weniger vollkommene Reife und Ausbildung des Kornes, sein Alter, seine Heimath u. s. w. werden darauf Einfluss nehmen können, daher man mit der Angabe sich wird zufrieden stellen müssen, dass für irgend eine beliebige Samenart die obere Grenze zwischen diesen und jenen Graden schwankte und entweder näher der einen oder der anderen Zahl liege."

Course of germination at maintained temperatures

Although it is interesting to note that the graphs of figure 2, which represent the performance of a population of seeds subdivided into cultures that were incubated at different temperatures, bear a superficial resemblance to many temperature graphs of physiological processes, such as growth, yet it must be remembered that the germination percentages of this study do not refer to process rates in individual seeds. The percentages are statistical values representing the various proportions of a seed population that were able to attain a specified developmental stage in cultures of certain ages at the specified temperatures. These proportions are dependent, not only upon prevailing environmental conditions and the duration of incubation, but also upon differences in physiological behavior among the individual seeds constituting the population. The percentage values provide a means for analyzing the physiological variability of the individual seeds within the population and they will now be considered with reference to several types of analysis designed to reveal some features of the nature and extent of this diversity.

In the graphs of figure 3, germination percentages are represented as ordinates and lengths of incubation time are abscissae, the data for each temperature being plotted separately. The form of each graph shows how the original seed population became gradually transformed, with lapse of time, into a population of seedlings. For all five tested temperatures this transformation evidently followed the same general course. All five graphs are sigmoid and their respective apparent points of inflection are indicated by circles. In general, the cumulative germination percentage increased slowly during the earlier observation periods, and then more rapidly, until a percentage corresponding to the apparent point of inflection of the graph was reached, after which the rate of increase fell off more and more. The five graphs are so similar that if they were plotted as arising from a common abscissa they would nearly coincide throughout. One feature of their close similarity is well shown by the fact that the percentage values for their apparent points of inflection all fall within the narrow range from about 36.4 to about 39.9.

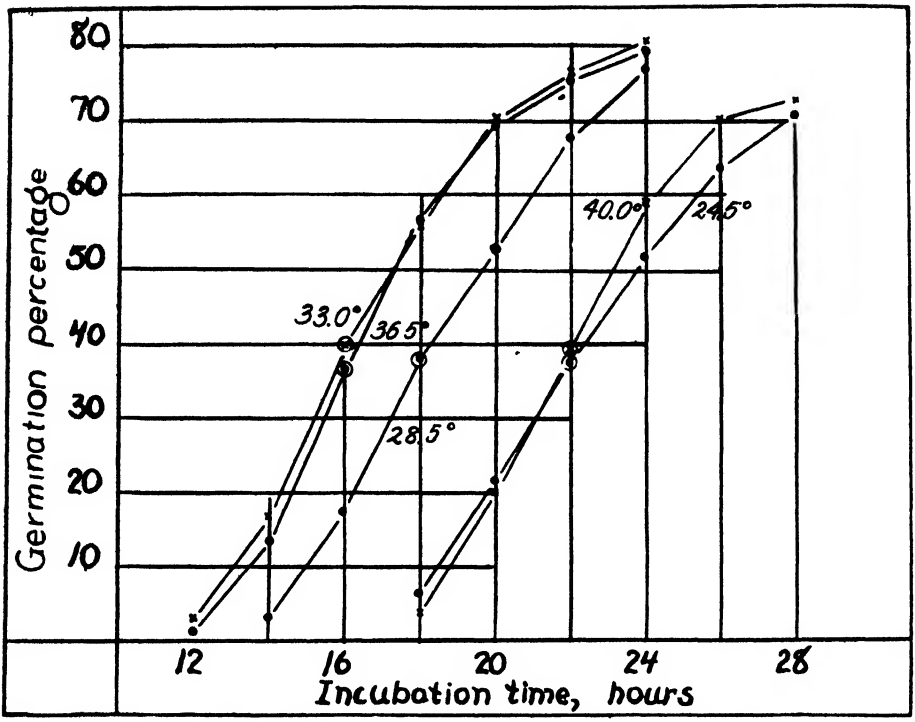


FIG. 3. Graphs for Black Eyebrow soy bean seed showing germination-percentage values as related to incubation time. Each graph represents a different temperature. Approximate points of inflection shown by circles.

The principal differences among these graphs are with respect to the time periods they represent. The graphs for 33.0° and 36.5° are seen to be almost identical in this regard and those for 24.5° and 40.0° are not greatly divergent, but the graph for 28.5° stands by itself. Less than 12 hours of incubation at 33.0° or 36.5° sufficed for the appearance of the first seedlings; in cultures at 28.5° the first seedlings were observed only after 14 hours of incubation; and seedlings did not begin to appear in cultures at 24.5° or 40.0° until these had been incubated for 18 hours. This same relationship between incubation time and temperature persisted throughout this study; any given percentage value was attained in cultures at 33.0° or 36.5° about two hours earlier than in cultures at 28.5°, and about six hours earlier than in cultures at 24.5° or 40.0°.

This kind of graph, showing progress of seed germination as related to lapse of time, has not been employed by many writers in this general field; but many papers give tabular data from which graphs of this sort may be constructed, and such graphs would generally be similar in their essentials to those shown in figure 3. Among the publications in which special

attention is given to this kind of analysis may be mentioned HARRINGTON'S (16, p. 348) germination-time graphs for his lot of seed of *Impatiens balsamina,* which present important relations and do not differ in general form from those of figure 3. The graphs given by EVANS (7), derived from seed of *Amaranthus retroflexus* germinated at nine different temperatures, show more variability in form than those of figure 3. A temperature of 42° gave the highest time rate of seedling production. The first seedlings appeared later in cultures at 46.1° than in those at 42°, and the cumulative increase in germination percentage was slower in cultures at 46.1°.

INCUBATION TIMES CORRESPONDING TO REPRESENTATIVE PERCENTAGE VALUES

As an alternative to representing the data of table I in terms of germination percentages attained after incubation periods of definite length, it is possible by interpolation to obtain from these data the lengths of time that correspond to certain definite germination percentages. The results of such a series of interpolations are shown by the graphs in figure 4, in which lengths of incubation time are measured on the vertical axis while temperature is indicated on the horizontal axis. There are three graphs, for 3, 38, and 70 per cent. respectively. These particular values are selected for special study because mean percentages of about these magnitudes appear for several temperatures in the table, and because the percentage value 38 is especially interesting since it represents the proportion of seeds that had germinated before the rate of seedling production began to fall off (see figure 3, the points of inflection). The graphs of figure 4 are concave upward, the low-temperature ends of the ones for 3 and for 70 per cent. being farther apart than the high-temperature ends. This difference between the lengths of time corresponding to these two percentages decreases as higher temperatures are examined, and it is interesting to note that there is no evident alteration of this relation as the optimal range of temperatures is approached and passed. These graphs suggest that an optimal range of temperature for seed germination might be taken as the range for which the least time is required for the germination of a specified proportion of the lot of seed studied. Thus the temperature range 33.0°–36.5° might be considered as optimal for the percentage range 3–70, according to the graphs of figure 4.

These graphs may be viewed as contour lines on a 3-dimensional diagram showing the relations of temperature, incubation time, and germination percentage. For each germination percentage many different lengths of incubation period are each seen to have two separate temperature ranges equally favorable for seedling production, one of these being suboptimal and the other supraoptimal.

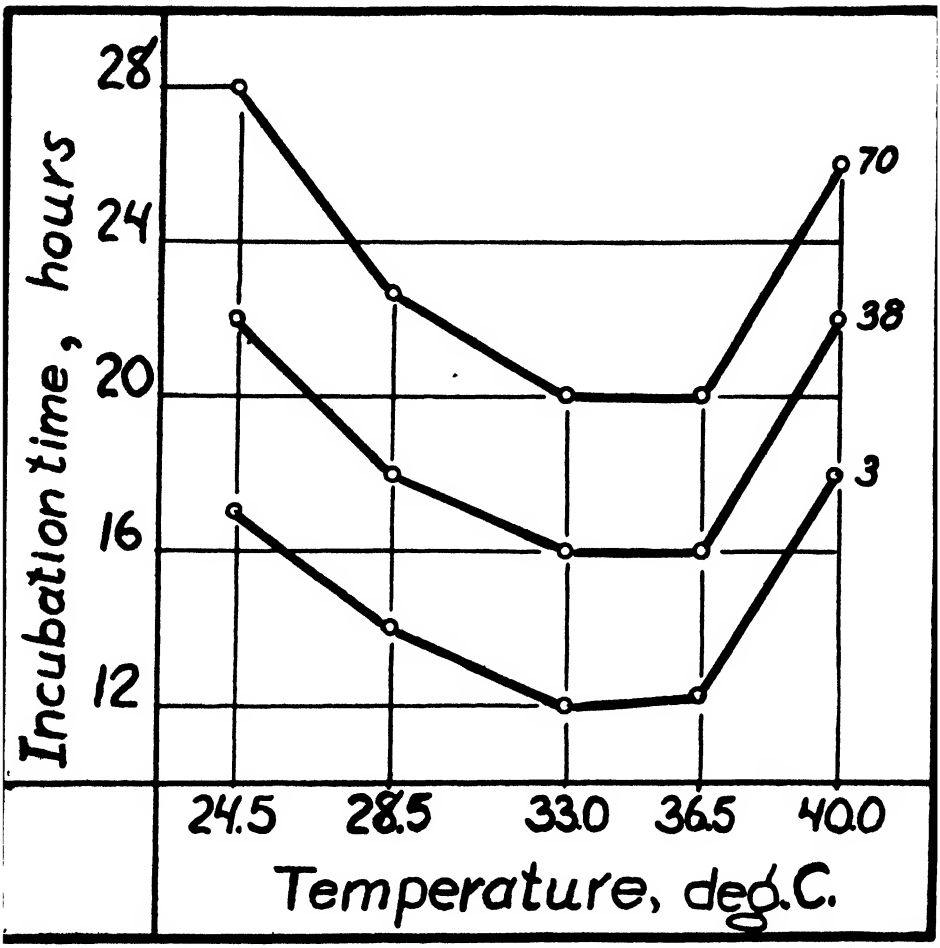


FIG. 4. Graphs showing relation of temperature to length of incubation time required for germination of specified percentages of Black Eyebrow soy bean seed. Each graph represents a different germination percentage.

Similar relations of incubation time to temperature have been reported by several workers. In the course of experiments on temperature relations of nuclear phenomena, HARTMANN (17) made some preliminary germination tests at eight constant temperatures between 8.5° and 41.0°, using moist sawdust as substratum. For seed of *Phaseolus multiflorus, Helianthus annuus,* maize, and pea, 31° induced the earliest appearance of seedlings; at higher and lower temperatures seedlings appeared later. BREMER'S (3) results for germinating beet seed show an optimal temperature range (22°–36°) for which the production of seedlings required only one or two days of incubation; with higher and lower temperatures longer

incubation was necessary. REYNOLDS (32), KOTOWSKI (21), WILSON and HOTTES (41), and WILSON (40) noted a diminution in the incubation period necessary for germination as higher temperatures were examined, but these tests, which embraced no temperatures above 30°–32°, did not involve temperatures high enough to induce retardation of germination. HABERLANDT (13, 14), KREYSING (22), and BÄR (2) also found definite evidence of a retardation in seedling production at high temperatures as compared with lower ones. LIVINGSTON and HAASIS's (24) graphs showing the relations of temperature, incubation period, and germination percentage for their French rice seed are similar to the graphs of figure 4, but the range of period length between the beginning of germination and the attainment of 90 per cent. is shown to be narrower for the optimal temperature (about 34°) than for suboptimal or supraoptimal temperatures.

INCREMENTS OF GERMINATION PERCENTAGE

It is evident that the seeds tested in this study varied considerably in rate of response to uniform environmental conditions, because if all the seeds had possessed identical physiological properties every radicle should have emerged after the same period of incubation at any given temperature. Although indications as to the nature of the diversity existing within a seed population may be obtained by inspection of sets of graphs such as those of figure 3, some features of this diversity may be more readily appreciated if the increments of germination percentage from observation to observation are employed. Such percentage increments, computed from the data of table I, are set forth by means of the histograms of figure 5, in which the 2-hour observation intervals of the incubation period are indicated on the horizontal axis while the increments in question are plotted vertically, each of their values being inscribed just above its horizontal segment of the graph. This set of graphs thus constitutes a table of values arranged in graphic form. Each of the five temperatures is represented by a separate graph.

Increments of germination percentage may be treated in either of two ways. (1) Attention may be focused on the fluctuating rate at which the original seed population is transformed into a seedling population and the average rates for the several 2-hour intervals may be compared to bring out acceleration and retardation. Since counts of germinated seeds were made at each observation, these rates for the several intervals are to be read directly (as percentages of the seed population) from the graphs of figure 5. Or (2) the percentage increments may be considered as measures of one kind of physiological diversity which was latent in the seed population. The histograms of figure 5 thus become frequency polygons that show

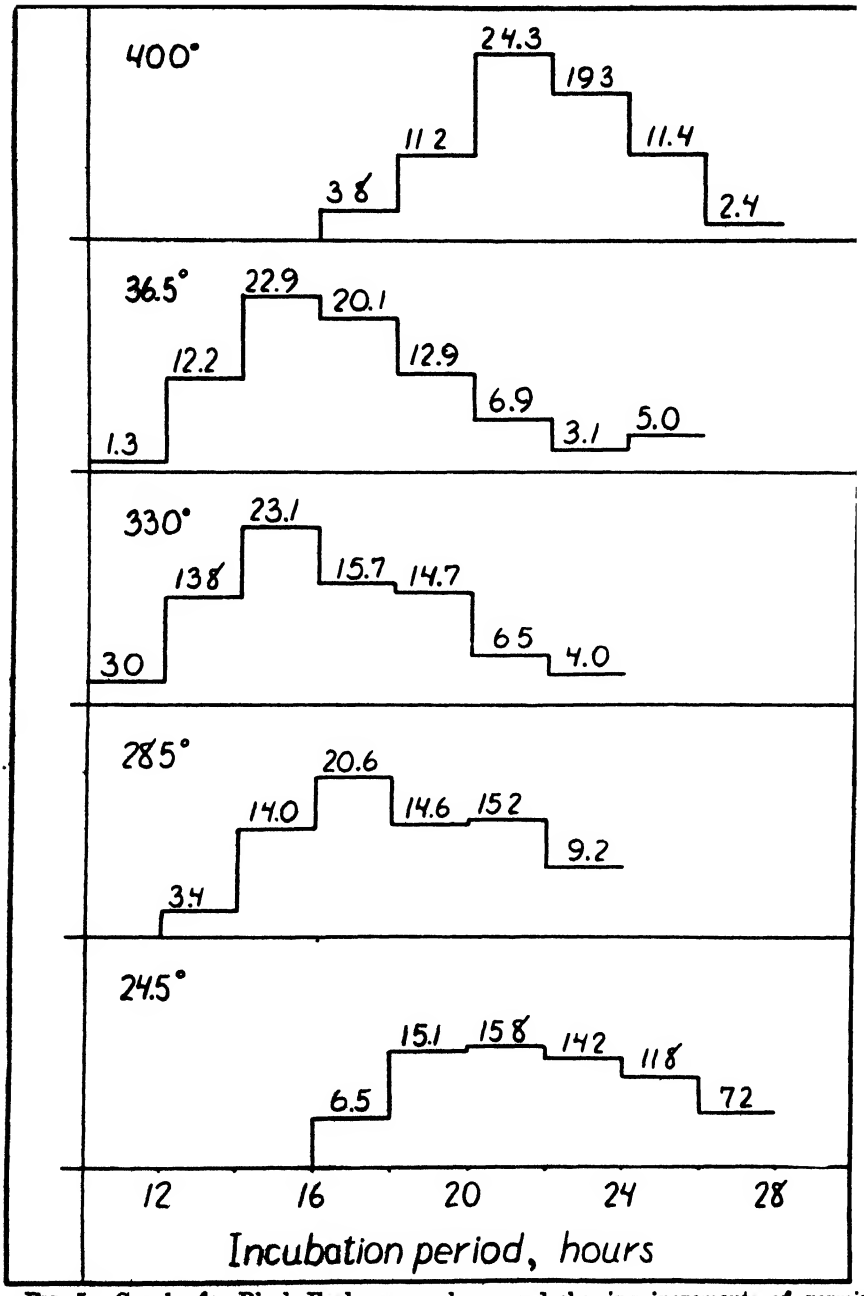


FIG. 5. Graphs for Black Eyebrow soy bean seed showing increments of germination percentage for 2-hour intervals. Each graph represents a different maintained temperature.

the relative proportions of the population that were embraced in several physiological classes of seeds. Since both these modes of treatment are instructive, the 2-hourly increments of germination percentage will be discussed in terms of each, in turn.

RATES OF SEEDLING PRODUCTION.—The rate of seedling production is seen (fig. 5) to have changed in a rather regular way for each of the five temperatures tested. Beginning with a low value for the first interval that yielded any seedlings at all, it increased rapidly to a maximum, shown for the third 2-hour interval of seedling production, and then it decreased. For the three lower temperatures the rate for the fourth interval is about like that for the second, and for the two higher temperatures this is true of the rate for the fifth interval. For subsequent intervals the rate generally continued to decrease progressively. The decrease is usually seen to have been less rapid than the earlier increase; *i.e.*, retardation of the rate of seedling production was less rapid than acceleration. The differences between successive values of the rate are more marked for the higher temperatures than for the lower ones; for 24.5° there is relatively little difference between the 18th hour of incubation and the 26th hour, and no doubt this tendency toward uniformity of rate throughout many consecutive 2-hour intervals would be still more pronounced if still lower temperatures had been employed. There was evidently a direct relation between the incubation temperature and the maximum rate of seedling production, the rate for the third interval after seedlings began to appear being greater as the temperature was higher

Since the peaks of the graphs of figure 5 mark the respective points at which seedling production began to fall off, the abscissa of the peak for each temperature is equivalent to the abscissa of the apparent point of inflection on the corresponding graph of figure 3. Both figures consequently show the same relations between incubation time and the maximal rate of seedling production for each tested temperature, for the rate of seedling production is of course just the rate of increase in the cumulative germination percentage in each series. Table II shows these relations.

Although the maximal 2-hour rate of seedling production and the duration of incubation required for the development of that maximal rate are both seen to have varied considerably and consistently with the degree of temperature employed, yet the total germination percentage corresponding to the apparent points of inflection on the graphs of figure 3 (and hence to the maximal rates of seedling production) appears to have shown no significant and consistent variation with temperature. This apparently critical percentage value is constant within the narrow range from 36.4 to 39.9, and its average magnitude for all five temperatures is 38.2. This is a remarkable constancy, and it is at once suggested that we have here a critical

TABLE II

MAXIMAL 2-HOUR RATES OF SEEDLING PRODUCTION AND CORRESPONDING GERMINATION PERCENTAGES AND LENGTHS OF INCUBATION PERIOD AT DIFFERENT TEMPERATURES DERIVED FROM FIGURES 3 AND 5

MAINTAINED TEMPERATURE	MAXIMAL 2-HOUR RATE OF SEEDLING PRODUCTION (FROM FIG. 5)	GERMINATION PERCENTAGE CORRESPONDING TO MAXIMAL RATE OF INCREASE IN GERMINATION PERCENTAGE (FROM FIG. 3)	INCUBATION PERIOD
°C.	%	%	hr.
24.5	15.8	37.4 ± 2.2	22
28.5	20.6	38.0 ± 2.0	18
33.0	23.1	39.9 ± 0.5	16
36.5	22.9	36.4 ± 1.2	16
40.0	24.3	39.3 ± 2.2	22

index of some characteristic or combination of characteristics of this lot of seeds. Apparently about 35–40 per cent. of the individuals were able to germinate with exceptional promptness, no matter which of the five widely different temperatures was employed. Whether the group of specially prompt and highly temperature-tolerant seeds (with respect to their capacity for germination) would embrace the same individuals for all favorable test temperatures is of course not known, for this apparently significant percentage is merely a statistical value pertaining to the entire lot.

Such an index may perhaps be of value in practical seed testing, for useful viability indices of stocks of seed may well refer definitely to capacity for prompt (although incomplete) germination of representative samples under a variety of environmental conditions, rather than to capacity for ultimate (and more nearly complete) germination of the samples with an environmental complex that is specially favorable to germination without reference to promptness. Several investigators mentioned by TOUMEY (37, p. 128) have recognized that the percentage of seeds germinating before the rate of seedling production begins to fall off may be used to represent the capacity of a given lot of seeds to produce seedlings quickly. In many situations encountered in forestry and agriculture, plant competition is so severe that an early start may be essential if a plant is to survive. TOUMEY used the name "germinative energy" for this percentage value, a term not wholly satisfactory, since ambiguity in the broader usages of science is apt to arise when a word (such as energy) with a definite meaning in one field (physics) is used in a different sense in another field (biology).

PHYSIOLOGICAL CLASSES OF SEEDS.—If attention be directed to the time spans covered by the process of extrusion of radicles, the degree of diver-

sity of individual seeds with respect to rate of response to the prevailing germination conditions becomes more apparent. For instance, the first seedlings were found after 12 hours of incubation at 33.0°, and after 24 hours only 80 per cent. of the population had germinated. It seems evident that there must have been important physiological differences of some kind among the seeds of this lot, when, for example, some seeds required only 12 hours and others required 24 hours to reach the same stage of development, although all were treated alike.

Since the cultures were examined every two hours and all germinated seeds were removed at each examination, this procedure operated as a sorting process, the seeds falling into one or another of several different physiological classes. Each class was characterized by rates of response of its members to specified conditions of incubation. If the histograms of figure 5 are examined with this concept in mind, then the intervals on the horizontal axis represent the various physiological classes into which the seeds were sorted, and the height of each segment or block of the histogram indicates the relative size of its particular class. Comparison of the five histograms brings out some notable similarities. Each shows a tendency for the values for length of incubation time to fall close to a crude mode (the third interval measured from the beginning of the appearance of seedlings), and the farther removed a class is from the crude mode the fewer are the individuals in it. Furthermore, the number of individuals with longer incubation periods than the modal length exceeds the number with shorter incubation periods, and each graph is consequently somewhat asymmetrical. In these respects the histograms are not unlike many other frequency polygons representing the distribution of some quantitative character among the members of a population of organisms. In connection with the study here reported, similar data were obtained for several additional kinds of seed, including three soy bean varieties and *Alisma plantago-aquatica* L.; in nearly all instances where large seed samples were employed with a maintained temperature, a frequency polygon essentially much like those shown in figure 5 could be constructed from the experimental results. Perhaps the most remarkable feature of these histograms representing a grouping of the seeds into physiological classes is their evident similarity with respect to the size relations of the several classes. That this sort of graph takes so nearly the same form for all five temperatures renders this relation much more striking and apparently more significant than it might be if all tests had been carried out at the same temperature.

It will be observed that the higher-temperature graphs of figure 5 exhibit more abrupt steps or gradations from class to class than are shown by the lower-temperature graphs. This is probably related to the fact that the processes of germination went forward more rapidly at higher tempera-

tures than at lower ones. Obviously the results of this kind of sorting should depend not only on the statistical distribution of the different kinds of individuals in the original seed sample, but also on the time-temperature characteristics chosen for the sorting procedure. For example, if it were required to sort 80 per cent. of the seeds of a sample into a specified number of classes, and if the relative sizes of the several classes were also specified, then this sorting technique might be applied by using any one of a number of different maintained temperatures, but the observation intervals would need to be shorter for higher temperatures than for lower ones.

Probably the properties of the agar gel on which the seeds rested changed during incubation, and the slowly developing seeds may not have been subjected to exactly the same environmental conditions as the rapidly developing ones. If it had been possible to maintain the environmental conditions constant throughout the period of incubation, perhaps the rates of seedling production might have been somewhat different. If greater changes in the medium took place at high temperatures than at low ones, some of the differences in form of the graphs of figure 5 might be accounted for. Such considerations as these, however, cannot alter the significance of the fact that the rates of response of the individual seeds were not alike, for all the seeds of any culture received similar treatment at the start.

Although many students of seed germination have been aware that counts made at intervals in a germination experiment furnish data resolvable into graphs similar to those of figure 5, nevertheless this aspect of the general problem of germination relations has rarely been discussed. Perhaps one reason for this may be that many experimental studies on seed germination have been made with relatively small seed samples. With small samples the increments of germination percentage are naturally also small, and they are likely to be irregular as well. In the present study the seed samples were relatively large (from 800 to 3600 individual seeds per sample), and this seems to have insured for every sample a fair representation of all the physiological classes in the stock, smoothing out many irregularities of the component tests,—irregularities that might have obscured important relations if the samples had been much smaller.

EARLIER STUDIES ON PHYSIOLOGICAL CLASSES OF SEEDS IN SAME STOCK.—GOODSPEED (**11**) made daily germination counts on lots of seed from a great number of plants in his breeding experiments on tobacco, and showed how the daily percentage-increment values altered throughout a long experiment period. Recognizing that the regular removal of germinated seeds from the cultures results in a physiological classification, GOODSPEED proposed this as a method for analyzing the genetic constitution of the progeny of hybrid plants. His results refer to seed samples that were not very large (100–200 seeds per sample), and some of the irregularity in the relative magnitudes

of his increments may have been due to inadequate sampling. Expressed in the form of frequency polygons, his data take a variety of forms; most of the graphs show only one mode, but those for some samples of seeds from hybrid parents show two modes. A few are symmetrical and there is no apparent agreement among the others with regard to relative steepness on the two sides of the mode.

Although not concerned with seed germination, LOEB and NORTHROP'S (**25**) data on the temperature relations of the development of fruit flies (*Drosophila*) are of interest here. Flies were grown from eggs under controlled conditions at several constant temperatures between 10° and 31.5°. At regular intervals each culture was examined and record was made of the number of larvae present. Similar observations were subsequently made on pupae and on the emergence of imagoes. For each stage of development the same kind of individual variation was noted; the rate of appearance of individuals at any given developmental stage rose to a maximum and then fell off, usually more slowly than it had risen. Figure 6 shows the temperature-time relations of the emergence of *Drosophila* imagoes, the daily emergence-percentage increments for which have been computed from LOEB and NORTHROP'S tables. These histograms are plotted like those of figure 5 and require no special explanation. Rates of emergence are an expression of individual variation in capacity for development, and a study of this kind shows not only the constitution of the population under consideration but also the extent to which the units of the population are influenced by the various sets of conditions employed in the experimentation.

WAGGONER (**39**) studied the progress of germination in samples of radish seeds with various water contents, the samples having been subjected to a temperature of 80° for 30 minutes before being distributed to germinate on wet plaster-of-Paris blocks. Samples with original water content of 18 per cent. failed to show germination, and those with 14 or with 9 per cent. moisture showed limitation and retardation of germination as compared with the sample having water content of only 4 per cent. Daily counts of germinated seeds showed clearly the manner in which the time rate of seedling production increased to a maximum and then decreased. Histograms constructed from WAGGONER'S tables possess the same general characteristics as those shown in figure 5.

This germination method of separating physiological classes in a stock of seed has been used to advantage by CORRENS (**5**). Seeds from artificially pollinated plants of *Melandrium* (a dioecious form) were incubated on moist paper and the seedlings transplanted to soil as they appeared, being classified into quartiles on the basis of the time required for their emergence from the seed. Growing 11,558 of these seedlings to a developmental stage

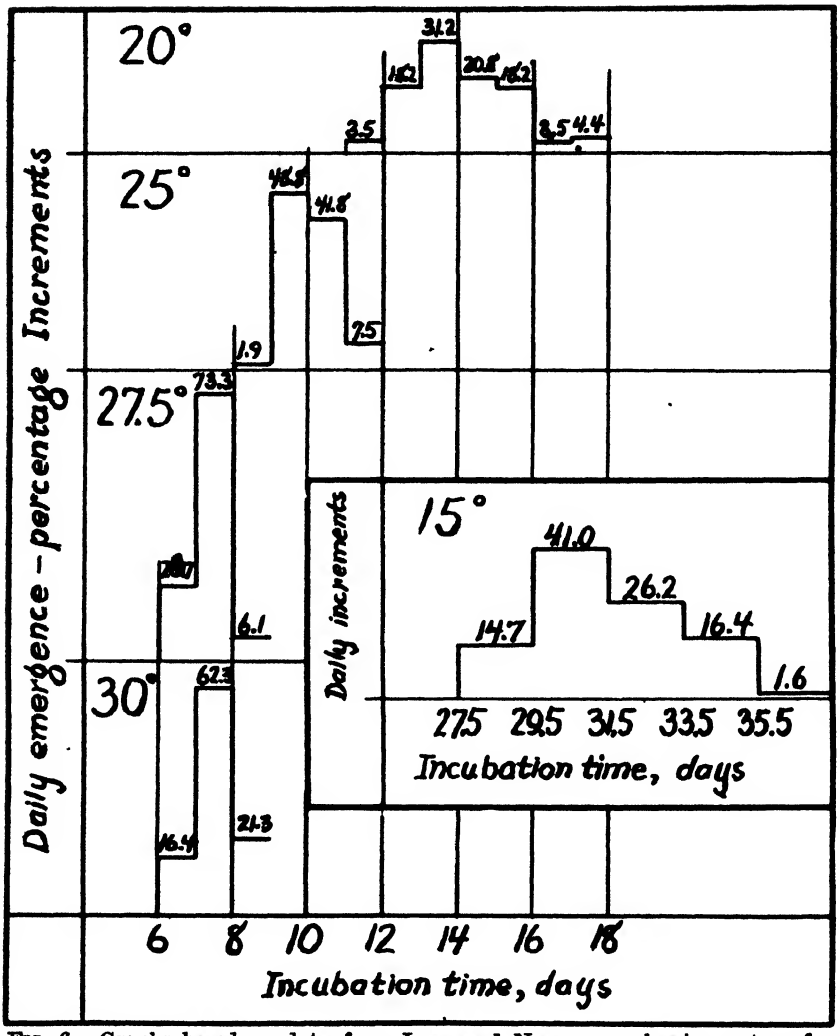


FIG. 6. Graphs based on data from LOEB and NORTHROP, showing rates of emergence of *Drosophila* imagoes at several maintained temperatures.

that permitted the staminate and pistillate individuals to be separately counted, CORRENS found that seeds destined to produce staminate plants tended to germinate more promptly than the others. In the progeny of one staminate parent the percentage of pistillate individuals in the first quartile (most rapid germination) was 32.8; in the second, 52.5; in the third, 62.6; and in the fourth (representing the seeds that had germinated most slowly), 69.0. Incidentally he observed that the rate of emergence

of seedlings rose to a maximum on the second or third day of incubation and declined thereafter.

PIEPER (**30**) observed that there was no correlation between the rate of germination of parents and of progeny in several samples of *Poa pratensis* seed from which he raised plants and obtained seed. He noted, however, that seeds from slowly germinating parents tended to germinate slowly, and that plants from rapidly germinating seeds produced seeds that tended to germinate rapidly.

PEARL (**28a**) observed differences in growth rate among seedlings sorted into physiological classes by methods similar to those used in the present study. After preliminary soaking, *Cucumis melo* seeds were sown on agar plates, incubated in darkness at 30°, and observed several times during the period of emergence of radicles. At each observation all germinated seeds were transferred to fresh agar plates and allowed to grow for an additional 48 hours. At the end of this growth period each group of seedlings was divided into cotyledons, hypocotyl, and roots, measurements of moist and fresh weight being made. The combined weight of hypocotyl and roots was taken as a measurement of growth. He found that the seeds which took the longest time in germinating made the least growth subsequently, and the best growth was made by the classes of seeds that germinated rapidly.

Both HAASIS (**12**) and TANG (**36**) called attention briefly to the possibilities of this sort of physiological technique for the sorting of a sample of seed into classes, but they gave only very limited attention to the question concerning the physiological classes of seedlings. The possibility of employing the temperature-time relations of seed germination as criteria in selecting similar lots of seedlings for physiological experimentation has been emphasized by LIVINGSTON and HAASIS (**24**).

Variation in the physiological characteristics of seeds may be manifested in other ways besides considering rates of seedling production. PORODKO (**31**) contributed information concerning the death rates at high temperatures, and SIFTON (**34**) reported on the death rates of seeds during storage. For each of these sets of data the decrement of the germination percentage may be studied and graphs similar to those of figure 5 may be constructed which bring out interesting relations. If, like HEWLETT (**18**), one suitably applies a toxic substance to a seed population for a given time period some of the seeds die, and these constitute a class whose individuals must have been characterized by about the same degree of resistance; if a suitably longer exposure period is used the seeds of still another class are killed, and so on. Naturally, if seeds must be killed in order to study their physiological variation, this method may be regarded as of limited usefulness in some respects; but the results secured by its application provide additional means

for comparing different lots of seed with respect to their intrinsic physiological variability.

Mean length of incubation time

Haberlandt (14), Pieper (29), Akemine (1), Gassner (9), and Kotowski (21) expressed the time relations of the germination of their seed samples by means of a mathematical expression or index that has considerable promise of usefulness when different lots of seed are to be compared. This expression is the mean length of incubation time required by the seed sample in question, for germination under specified test conditions. Kotowski used the reciprocal of the mean length of incubation time, calling it the "coefficient of velocity" of germination.

To derive the mean length of incubation time, a seed sample is tested in the manner employed in the present study, with frequent observations and records of the germination-percentage increments for the several observation intervals. Then each percentage increment is multiplied by the corresponding length of incubation period, measured from the beginning of the test, and all resulting products are summed. Finally, the sum thus secured is divided by the total germination percentage for the whole test; that is, by the sum of all percentage increments. For example, consider the percentage increments and the corresponding incubation times for the seeds of the present study, and for the maintained temperature 24.5° (fig. 5). The six increment-time products are $6.5 \times 18 = 117.0$, $15.1 \times 20 = 302$, $15.8 \times 22 = 347.6$, $14.2 \times 24 = 340.8$, $11.8 \times 26 = 306.8$, and $7.2 \times 28 = 201.6$. The sum of these products (1615.8) is divided by 70.6 (the sum of all six increments, or the final germination percentage) and the resulting quotient is 22.9 hours, which is the required mean incubation time for all tests at 24.5° carried out in this study. These individuals may therefore be said to have required, on the average, an incubation period of 22.9 hours for germination at 24.5° and under the background conditions of the experimental technique employed in these tests.

This mean has different values according to the five different maintained temperatures employed: for 24.5° it is 22.9 hours; for 28.5°, 19.4 hours; for 33.0°, 17.5 hours; for 36.5°, 18.1 hours; and for 40.0°, 22.8 hours. These values are slightly greater than the corresponding incubation times represented by the peaks of the graphs of figure 5, and when plotted their graph is very similar in form to the graphs of figure 4.

Because this index of germination varies with the maintained temperature used in the experiment from which it is derived, it may furnish an additional criterion for establishing a temperature optimum for the germination of a stock of seed, that temperature being taken as optimal for which the lowest mean incubation time is shown. Thus from the values

just given, it appears again that a temperature optimum may be considered as between 33.0° and 36.5°; the difference between the two index values 17.5 (33.0°) and 18.1 (36.5°) probably being insignificant.

When several stocks of seed are to be compared as to average promptness of germination, they might be tested severally in a manner similar to that of this study and a score number or coefficient of germination capacity for each stock might be the mean incubation time corresponding to the optimal temperature for that stock; *i.e.*, the shortest mean incubation times shown by the several stocks, or the reciprocals of these time values, might be useful indices for the comparison of different stocks. Many other uses for this mean incubation time might be suggested.

Summary

1. Samples of a stock of seed of Black Eyebrow soy bean were incubated on 0.5-per cent. agar plates at five maintained temperatures (24.5°, 28.5°, 33.0°, 36.5°, and 40.0°) and the number of germinated seeds recorded at 2-hour intervals, until about 80 per cent. of all the seeds of a test had germinated. According to several different criteria, the optimal temperature range for germination was found to be 33.0°–36.5°. Within this range (a) the least time was required for the appearance of any given germination percentage, (b) the greatest percentage of seeds germinated during any of the incubation periods tested, and (c) the mean incubation time was shortest.

2. The rate of seedling production, measured as the percentage of the total seed population germinating per 2-hour interval, showed a maximum for the third 2-hour interval in which any seedlings were found. For each of the five temperatures tested the germination percentage attained when the rate of seedling production began to fall off was found to be 35–40. For later incubation intervals the rate of seedling production decreased more slowly than it had increased prior to the attainment of its maximal value.

3. The removal of germinated seeds from test cultures at regular intervals constitutes a sorting process which distributes the seedlings derived from a seed sample into several classes, according to their rates of emergence under specified uniform and favorable sets or complexes of germination conditions. In terms of this concept, the increments of germination percentage from one observation to the next indicate the relative sizes of these physiological classes in the original seed sample as well as in the resulting lot of seedlings.

The writer wishes to express his appreciation to Professor B. E. Livingston, of Johns Hopkins University, where these experiments were con-

ducted, for his friendly advice and criticism concerning the preparation of this paper; thanks are also due to Professor RAYMOND PEARL for helpful criticism on the methods of analysis used.

JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

LITERATURE CITED

1. AKEMINE, M. Zur Kenntnis der Keimungsphysiologie des Reises. Fühlings Landw. Zeit. **63**: 78–93. 1914.
2. BÄR, KARL. Untersuchungen an Rot- und Schafschwingel. Jour. Landw. **76**: 129–150. 1928.
3. BREMER, H. Die Abhängigkeit der Zuckerrübenkeimung von der Temperatur. Angew. Bot. **11**: 112–115. 1929.
4. COLLINS, G. N. The application of statistical methods to seed testing. U. S. Dept. Agr. Circ. 79. 17 p. 1929.
5. CORRENS, C. Der Unterschied in der Keimungsgeschwindigkeit der Männchensamen und Weibchensamen bei Melandrium. Hereditas **9**: 33–44. 1927.
6. EDWARDS, T. I. The temperature relations of germinating seeds. Quart. Rev. Biol. **7**: 428–443. 1932.
7. EVANS, CLYTEE R. Effect of temperature on germination of *Amaranthus retroflexus*. Bot. Gaz. **73**: 213–226. 1922.
8. GARBER, R. J., and ODLAND, T. E. Natural crossing in soy beans. Jour. Amer. Soc. Agron. **18**: 967–970. 1926.
9. GASSNER, GUSTAV. Die Feststellung der Schädigung des Saatgutes durch Beizmittel. Zeitschr. Pflanzenkrankheiten und Pflanzenschutz **36**: 25–41. 1926.
10. GERICKE, W. F. Some relations of maintained temperatures to germination and the early growth of wheat in nutrient solutions. Philippine Jour. Sci. **38**: 215–238. 1929.
11. GOODSPEED, T. H. Notes on the germination of tobacco seed. II. Univ. California Pub. Bot. **5**: 233–248. 1915.
12. HAASIS, F. W. Germinative energy of lots of coniferous tree seed, as related to incubation temperature and to duration of incubation. Plant Physiol. **3**: 367–412. 1928.
13. HABERLANDT, FRIEDR. Die oberen und unteren Temperaturgrenzen für die Keimung der wichtigeren landwirtschaftlichen Sämereien. Landw. Versuchsst. **17**: 104–116. 1874.
14. ———. Die untere und obere Temperaturgrenze für die Keimung einiger Culturpflanzen wärmerer Klimate. Haberlandts wiss.-prakt. Unters. auf dem Gebiete des Pflanzenbaues **1**: 117–122. 1875.

15. HAFENRICHTER, A. L. Respiration of the soy bean. Bot. Gaz. **85**: 271–298. 1928.
16. HARRINGTON, G. T. Optimum temperatures for flower seed germination. Bot. Gaz. **72**: 337–358. 1921.
17. HARTMANN, OTTO. Über den Einfluss der Temperatur auf Plasma, Kern und Nucleolus und cytologische Gleichgewichtszustände. Zellphysiologische Experimente an Pflanzen. Arch. Zellforschung **15**: 177–248. 1919.
18. HEWLETT, R. T. Disinfection and disinfectants. Lancet **1**: 889–894. 1909.
19. JONES, F. R., and TISDALE, W. B. Effect of soil temperature upon the development of nodules on the roots of certain legumes. Jour. Agr. Res. **22**: 17–31. 1921.
20. KIDD, FRANKLIN, and WEST, CYRIL. Physiological pre-determination. The influence of the physiological condition of the seed upon the subsequent growth and upon the yield. Ann. Appl. Biol. **5**: 1–10; 112–142; 157–170; 220–251. 1918–19. *Ibid.* **6**: 1–26. 1919.
21. KOTOWSKI, FELIX. Temperature relations to germination of vegetable seed. Proc. Amer. Soc. Hort. Sci. **1926**: 176–184. 1927.
22. KREYSING, MAX. Beiträge zur Keimungsphysiologie des Wiesenschwingels. Jour. Landw. **72**: 237–278. 1924.
23. LIVINGSTON, B. E., and FAWCETT, H. S. A battery of chambers with different automatically maintained temperatures. Phytopathol. **10**: 336–340. 1920.
24. ————————, and HAASIS, F. W. Relations of time and maintained temperature to germination percentage for a lot of rice seed. Amer. Jour. Bot. **20**: 596–615. 1933.
25. LOEB, JACQUES, and NORTHROP, J. H. On the influence of food and temperature on the duration of life. Jour. Biol. Chem. **32**: 103–121. 1917.
26. MORSE, W. J. Soy beans; culture and varieties. U. S. Dept. Agr. Farmers Bull. no. 1520. 1927.
27. NIETHAMMER, ANNELIESE. Stimulationsprobleme in Zusammenhang mit den inneren Faktoren, die Keimung bedingen. Beitr. Biol. der Pflanzen 16: 267–350. 1928.
28. OATHOUT, C. H. The vitality of soybean seed as affected by storage conditions and mechanical injury. Jour. Amer. Soc. Agron. **20**: 837–855. 1928.
28a. PEARL, RAYMOND. On the distribution of differences in vitality among individuals. Amer. Nat. **61**: 113–131. 1927. Also published in The Rate of Living, pp. 97–121. New York. 1928.

29. PIEPER, H. Zur Methode der Keimprüfung. Jour. Landw. **57**: 137–147. 1909.
30. ————. Über die Erblichkeit der Keimgeschwindigkeit, der Keimfähigkeit und der Lichtempfindlichkeit der Samen von *Poa pratensis*. Fühlings Landw. Zeitung **58**: 362–368. 1914.
31. PORODKO, TH. M. Über die Absterbegeschwindigkeit erhitzten Samen. Ber. deutsch. bot. Ges. **44**: 71–80. 1926.
32. REYNOLDS, J. B. Temperature in relation to seed germination. Ann. Rept. Dept. Agr. Prov. Ontario **1903**: 9–11. 1904.
33. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
34. SIFTON, H. B. Longevity of the seeds of cereals, clovers, and timothy. Proc. Assoc. Offic. Seed Analysts of N. Amer. **1921**: 22–24. 1921.
35. STARK, O. K. The protein metabolism of the soy bean. Amer. Jour. Bot. **14**: 532–547. 1927.
36. TANG, PEI-SUNG. An experimental study of the germination of wheat seed under water as related to temperature and aeration. Plant Physiol. **6**: 203–248. 1931.
37. TOUMEY, J. W. Seeding and planting. New York. 1916.
38. VON OHLEN, F. W. A microchemical study of soy beans during germination. Amer. Jour. Bot. **18**: 30–49. 1931.
39. WAGGONER, H. D. The viability of radish seeds (*Raphanus sativus* L.) as affected by high temperatures and water content. Amer. Jour. Bot. **4**: 299–313. 1917.
40. WILSON, H. K. Wheat, soy bean and oat germination studies with particular reference to temperature relationships. Jour. Amer. Soc. Agron. **20**: 599–619. 1928.
41. ————, and HOTTES, C. F. Wheat germination studies with particular reference to temperature and moisture relationships. Jour. Amer. Soc. Agron. **19**: 181–190. 1927.

FACTORS AFFECTING ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN, PARTICULARLY IN TOMATO AND APPLE

VICTOR A. TIEDJENS[1]

(WITH SIX FIGURES)

Introduction

Plants absorb ammonium and nitrate nitrogen in varying quantities, depending partly on the rate of assimilation[2] of either ion in the plant. That certain specific conditions are necessary for the most efficient assimilation of either form of nitrogen has been pointed out (**40**). Different species vary in the efficiency with which they utilize the nitrate or ammonium ion under a given set of conditions (**29**). The hypothesis that both ions probably are finally utilized as ammonium, even though external requirements may be different, involves an interesting study, a preliminary report of which has been published (**40**). A continuation of these studies has established certain relationships affecting the assimilation of nitrate and ammonium nitrogen. Such factors as hydrogen-ion concentration and available carbohydrates have a direct bearing on the assimilatory processes. That these relationships vary with the source of nitrogen, and that the location of the initial assimilatory processes varies with the type of plant, will be pointed out.

Experimental methods

Tomato and cotton plants, and small 1-year-old Delicious, Stayman, and Baldwin apple trees, all root-grafted, were grown in sand cultures. The nutrient solutions were modifications of one of the ammonium sulphate series recommended by JONES and SHIVE (**12**), the compositions of which are shown in table I.

Calcium and sodium salts were used as the source of nitrate, whereas ammonium sulphate, ammonium carbonate, and ammonium hydroxide were used as the sources of ammonium. Details of growing the plants for some of the experiments have been discussed elsewhere (**39, 40**).

Experiments were conducted with the tomato (*Lycopersicon esculentum* Mill.) cotton, and apple (*Pyrus malus* L.) in which the pH of the solutions percolating through the sand was maintained within 0.2 of the initial pH.

[1] The writer acknowledges his indebtedness to G. T. NIGHTINGALE, SANTE MATTSON, and J. W. SHIVE for their helpful suggestions during the progress of the experiments and the preparation of the manuscript.

[2] Assimilation in this report refers to the reduction and elaboration of the nitrate and ammonium ions after they have been absorbed by the plant.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATION OF COMPOUNDS IN NUTRIENT SOLUTIONS. INITIAL pH OF NUTRIENT SOLUTIONS WAS MODIFIED TO SATISFY REQUIREMENTS OF EXPERIMENTS WITH 0.1N SULPHURIC ACID OR POTASSIUM HYDROXIDE UNLESS OTHERWISE STATED

SERIES	pH*	KH_2PO_4	$CaCl_2$	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	$NaNO_3$	$MgSO_4 \cdot 7\,H_2O$
A	3–9 incl.	0.00633	0.00292	.....	0.00584	.....	0.00237
B	3–9 incl.	0.00633	0.00292	.....	.....	0.01168	0.00237
C	4–9 incl.	0.00633	0.00292	0.00560	.....	.....	0.00237
D*	6.4–9 incl.	0.00633	0.00292	.....	.....	.....	0.00237
E	8.0	0.00530	0.00146	0.00840	.....	.....	0.00118
F	8.0	0.00633	0.00584	0.00140	.....	.....	0.00237
G	5.1	0.00633	0.00584	.....	0.00146	.....	0.00237

* Number of cc. of $\frac{M}{2}$ ammonium hydroxide per liter for each pH as indicated: pH 6.4 = 1, 6.8 = 2, 7.3 = 3, 7.8 = 4, 8.4 = 6, and 8.8 = 8 cc. The pH 8.4 cultures did not receive quite so much nitrogen as the C cultures supplied with ammonium sulphate.

In these cultures from 10 to 24 liters of nutrient solution, varying with the size of the plants, were supplied to the sand cultures every 24 hours. This was accomplished by running 5 to 12 liters of solution through in 12 hours. The solution after dripping from the cultures was adjusted to the desired pH and used several times. The solutions were changed every third day. Solution lost by evaporation and transpiration was replaced by fresh nutrient solution. The osmotic concentrations varied slightly during the 3-day period that the solutions were used. Because of the large amount of nutrient solution required, the experiments necessarily were limited in scope, but were sufficiently comprehensive to establish the constant pH at which the plants would grow with nitrate and ammonium nitrogen, and supplemented those experiments from which the data have been published (**39, 40**).

EXTRACTION OF PLANT TISSUES BY CHEMICAL METHODS.—Two methods were employed in obtaining an extract of ionizable substances from the plant tissue, namely, aqueous extraction of the fresh tissue, and extraction of the fresh tissue by electrodialysis, which separated the anions from the cations into two separate solutions. When time did not permit immediate extraction of the fresh tissue, it was weighed into quart Mason fruit jars, sealed, and stored at -6 to $-20°$ C. until analyzed. The freezing method employed has been found to be satisfactory for this purpose (**24**). Handling the tissue in this manner made it possible to harvest an entire series of twenty or more samples and analyze them as time permitted.

EXTRACTION OF PLANT TISSUE BY ELECTRODIALYSIS.—The three-compartment electrodialyzer as employed and described by MATTSON (**16**) was found satisfactory for obtaining extracts of plant tissue. Cations were removed by a copper cathode and anions were removed by means of a platinum anode. Most of the amino acids, amides, and polypeptide groups were collected in the cathode chamber. An advantage of this method of extraction was the separation of nitrate nitrogen from the other nitrogenous fractions, thus eliminating nitric acid, which apparently interferes with the analysis of other nitrogenous fractions (**39**). Electrodialysis also left the more complex proteins in the central chamber. The solution containing the cations was usually clear, but in some cases contained a yellow pigment. The solution containing the anions was clear and usually colorless. Ammonium and other monovalent cations were extracted from the tissue in a very short time, whereas the anions were removed much more slowly. This has been shown by MOORE, REEVES, and HIXON (**19**). In the present studies it was found that ammonium ions were almost completely removed in 15 minutes.

The protein content in the tissue remaining in the central chamber was usually slightly lower in the electrodialyzed sample, and the soluble organic

and inorganic nitrogen was higher than in the residue of sample extracted with water and coagulated with dilute acetic acid. This was accounted for by difference in the efficiency of the two methods in extracting nitrates and ammonium ions, which will be shown by data to be published later. Amide nitrogen was entirely removed only after carefully spacing the electrodes and removing the dialysate from the cathode chamber at frequent intervals. Amides and amino acids will take on a positive or negative charge, depending on the pH of the solution in which they are dissolved, and will then tend to move to the pole of opposite charge. Asparagine has

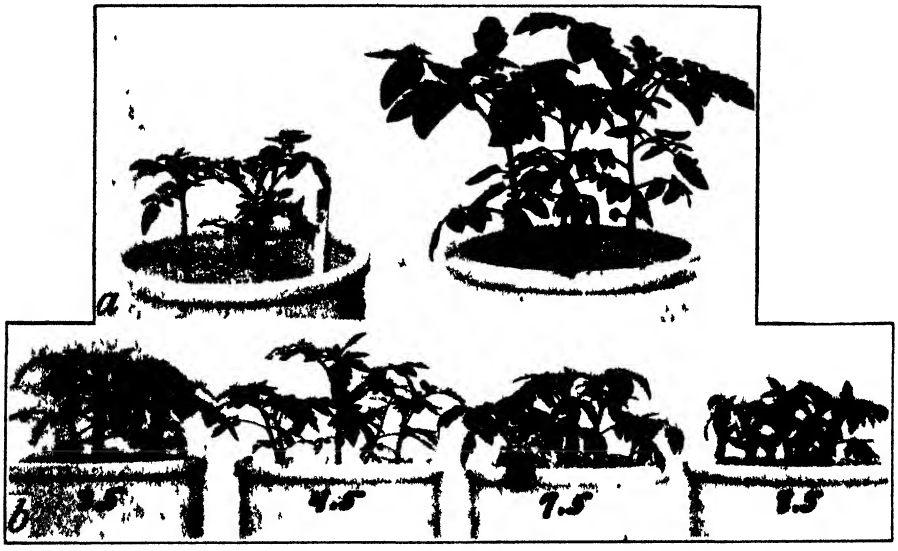


FIG. 1. *a*, tomato plants of experiment I in sand cultures, initially extremely high in carbohydrates, after 3 weeks with a complete nutrient solution containing sodium nitrate at initial pH 5.4 (left) and ammonium sulphate at initial pH 8.0 (right). *b*, tomato plants grown with sodium nitrate at initial pH 3.5 and 4.5 (left) and ammonium sulphate at initial pH 7.5 and 8.5 (right). These plants received the nutrient solution as soon as the first true leaf appeared.

an isoelectric point of pH 4.0 to 4.2. Asparagine in tomato sap at pH 5.4 probably has a negative charge; but when placed in the electrodialyzer, because of the rapid extraction of the monovalent cations, the material containing the asparagine in the central cell becomes very acid, causing the asparagine to take on a positive charge. It then tends to move in the direction of the cathode chamber, which by this time is definitely alkaline (above pH 8.0). Unless the solution from the cathode chamber is immediately removed, the charge on the asparagine is reversed and it will travel back to the central chamber. Amino acids with higher isoelectric points will

tend to remain in the cathode chamber longer, and are therefore more easily removed. Details regarding the application of the electrodialyzer for complete fractionation of the ionizable nitrogen will be presented in a separate paper. There are numerous advantages in extracting tissue in this manner, including the possibility of using colorimetric methods for estimating certain fractions.

AQUEOUS EXTRACTION OF PLANT TISSUE.—The fresh or frozen tissue was extracted with water and freed of protein by coagulation with dilute acetic acid after the method of CHIBNALL (**4, 5**).

The methods used for analysis of extracts have already been described (**39**).

Presentation of results

EXPERIMENT I.—Tomato plants were washed free of soil and set in sand when 4 inches high. They were given a minus-nitrogen solution until the stems were stiff and woody and the leaves yellowish green. Starch was abundant in all parenchymatous tissue. The plants were then divided into two series of twenty cultures each. One series was supplied with ammonium sulphate in a complete nutrient solution at pH 8.0, the other sodium nitrate at pH 5.1. The solution was not held at a constant pH (**38**). The roots, stems, and leaves were analyzed for nitrogenous fractions. Figure 1*a* shows these plants 3 weeks after being given nutrient solutions B and C (table I). Three weeks later, on October 15, when the plants of both series were about 10 inches high, they were harvested and analyzed. The chemical data are shown in table II and in columns 1 and 2 of figure 2.

The ammonium-supplied plants were definitely succulent, and had in the upper stem a very active cambium and starch (only in the region near the phloem). In the base of the stem there was still some starch in the pith. The tap root contained starch only in the endodermis. The plants which received nitrate had not become so succulent as the ammonium-supplied series and the cambium was not so active. Starch was distributed more generally throughout the plants with the exception of the tip. The dry matter of the roots and leaves, however, was only slightly higher. The plants of the ammonium series, as compared with those receiving nitrate, were more spreading, owing to softer petioles and slightly larger leaflets which had a tendency to droop more. The internodes, however, were slightly longer in the plants receiving nitrate.

EXPERIMENT II.—One lot of twelve tomato plants was grown to the fruiting stage with an abundant supply of ammonium sulphate, to determine how much organic nitrogen would accumulate, and whether it would be possible so completely to utilize carbohydrates that the plant might be injured as a result. A complete nutrient solution of which one-half of the

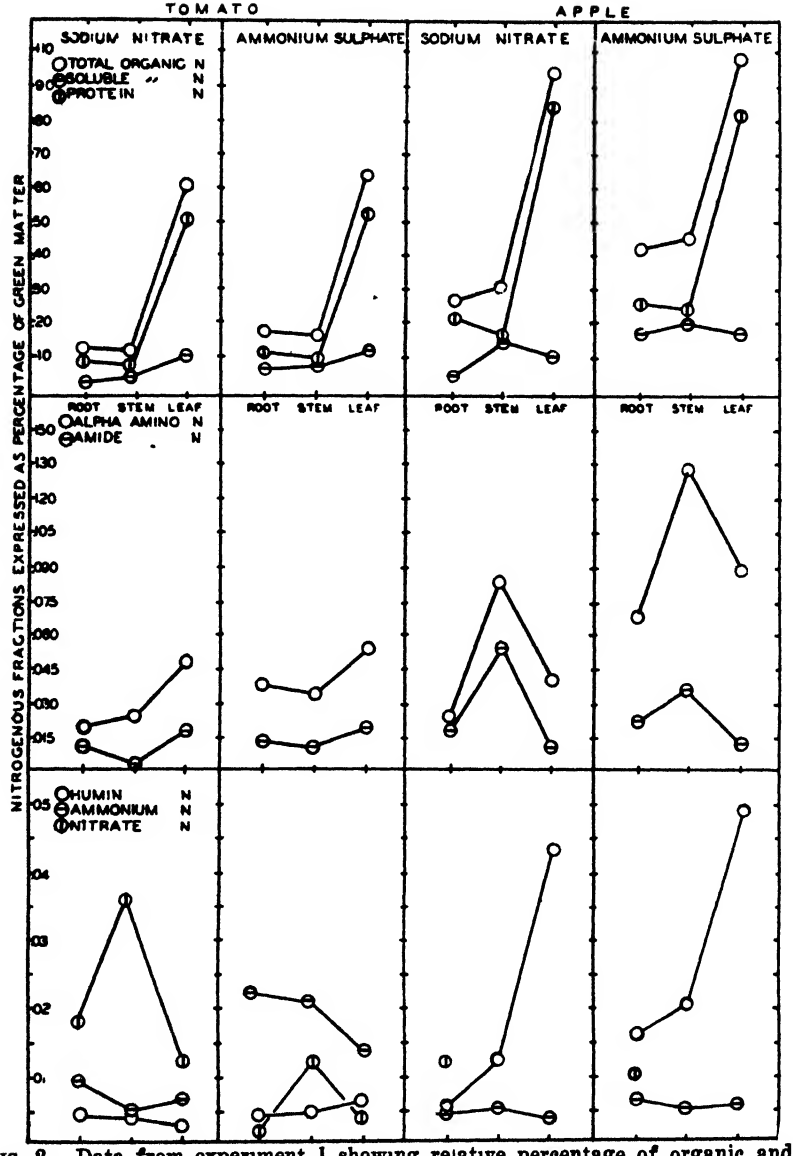


FIG. 2. Data from experiment 1 showing relative percentage of organic and inorganic nitrogen in ammonium and nitrate-supplied tomato. The comparable data on apple (experiment I) have been presented elsewhere (39) but are presented here for comparative purposes.

TABLE II

EXPERIMENT I

NITROGENOUS FRACTIONS IN CARBOHYDRATE HIGH TOMATO PLANTS SIX WEEKS AFTER BEING SUPPLIED WITH SODIUM NITRATE AT pH 5.1 OR AMMONIUM HYDROXIDE AT pH 8.0

	NITROGEN AS PERCENTAGE OF GREEN MATTER						PERCENTAGE OF TOTAL ORGANIC NITROGEN					
	ROOTS		STEMS		LEAVES		ROOTS		STEMS		LEAVES	
	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	8.0	6.0	7.0	8.0	13.0	12.0						
Total organic N	0.1217	0.1754	0.1187	0.1631	0.6040	0.6420	100.00	100.00	100.00	100.00	100.00	100.00
Protein N	0.0856	0.1095	0.0728	0.0919	0.5055	0.5255	70.34	62.43	61.33	56.35	83.69	81.85
Soluble organic N	0.0361	0.0659	0.0459	0.0712	0.0985	0.1165	29.66	37.57	38.67	43.65	16.31	18.15
Humin N	0.0045	0.0040	0.0037	0.0048	0.0025	0.0060	3.70	2.28	3.12	2.94	0.41	0.93
α Amino N	0.0162	0.0387	0.0221	0.0375	0.0443	0.0544	13.31	22.06	18.62	22.99	7.33	8.47
Amide N	0.0083	0.0130	0.0017	0.0118	0.0110	0.0194	6.82	7.41	1.43	7.23	1.82	3.02
Ammonium N*	0.0092	0.0221	0.0059	0.0218	0.0075	0.0135	6.32	11.07	3.68	11.03	1.20	2.05
Nitrate N†	0.0147	0.0022	0.0357	0.0127	0.0125	0.0045	10.10	1.10	21.60	6.43	2.00	0.68
Other N	0.0071	0.0102	0.0184	0.0171	0.0407	0.0367	5.83	5.82	15.50	10.49	6.75	5.72

* Percentage of total nitrogen.

† Percentage of total nitrogen (includes nitrate determined in extract from electrodialyzed tissue; none was recovered from ammonium plants by ordinary extraction).

TABLE III

Experiment II

Nitrogenous fractions as percentage of green matter in different parts of tomato plants which received ammonium sulphate as the only source of nitrogen in a complete nutrient solution supplied at initial pH 8.0 and final pH 3.8

	Plants harvested December 11											
	Fibrous roots		Stems		Old blades		Young blades		Old petioles		Young petioles	
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	9.68		10.13		9.2		10.4		7.03		6.4	
Total organic N	0.4800	100.00	0.3765	100.00	0.4230	100.00	0.6641	100.00	0.2171	100.00	0.2162	100.00
Protein N	0.2490	51.88	0.1443	38.33	0.2818	66.62	0.5003	75.34	0.0978	45.05	0.1045	48.34
Soluble organic N	0.2310	48.12	0.2322	61.67	0.1412	33.38	0.1638	24.66	0.1193	54.95	0.1117	51.66
Basic N	0.0296	6.17	0.0200	5.31	0.0405	9.57	0.0390	5.87	0.0195	8.98	0.0146	6.75
α Amino N	0.1211	25.23	0.1220	32.40	0.0561	13.26	0.0665	10.01	0.0617	28.42	0.0561	25.95
Amide N	0.0416	8.67	0.0275	7.30	0.0175	4.14	0.0190	2.86	0.0240	11.05	0.0346	16.00
Ammonium N*	0.0690	12.34	0.0450	10.55	0.0730	14.46	0.0550	7.58	0.0670	22.90	0.0430	16.21
Nitrate N†	0.0103	1.84	0.0050	1.17	0.0090	1.78	0.0065	0.90	0.0085	2.90	0.0061	2.30
Other N	0.0387	8.06	0.0627	16.65	0.0271	6.45	0.0393	5.92	0.0141	6.49	0.0064	2.96

* A large amount of this ammonia is combined ammonia (40).

† Ammonia and nitrate expressed as percentage of total nitrogen.

concentration was ammonium sulphate (E, table I) was supplied at pH 8. The ammonium sulphate was gradually increased from solution A to E as the plants grew larger. The plants were harvested December 11, at which time the days were short and relatively cloudy. The night temperature of the greenhouse during this period was kept between 56° and 62° F.

The plants were extremely succulent, although they grew slowly, and the leaves contained only 10 per cent. dry matter and practically no starch. The stems were more or less hollow, owing to disintegration of pith cells, and were comparatively soft. The plants were unfruitful, although they produced occasional flowers. There was no external indication of the high percentage of ammonium present. A trace of nitrate was detected throughout the plant. The nitrogenous fractions in the different plant parts are shown in table III.

EXPERIMENT III.—From April 20 to August 15, two series each consisting of twenty 1-year-old Delicious apple trees were grown in sand cultures. They were supplied with sodium nitrate at initial pH 5.1, and ammonium hydroxide at initial pH 8 (B and D respectively, table I). The chemical data and observations are reported in detail elsewhere (**39**), but for comparative purposes are summarized graphically in figure 2, columns 3 and 4.

EXPERIMENT IV.—One-year-old Delicious apple trees were set in nitrogen-free sand and grown with minus-nitrogen solution until all the inorganic nitrogen in the plants had been assimilated. They made from 12 to 15 inches of stem growth, the leaves became yellow and eventually abscissed, and the trees become dormant. These dormant trees were divided into two series of twenty each, were supplied daily with nitrate or ammonium solutions (B and D, table I), and were analyzed at intervals to determine the relative rates of assimilation of the two forms of nitrogen in dormant trees. The results have been published (**39**) but will be referred to in the discussion.

EXPERIMENT V.—One-year-old Delicious apple trees were placed in sand cultures and supplied with nutrient solutions containing ammonium sulphate or sodium nitrate at initial pH values ranging from 3.5 to 8.5 (B and C, table I). Ten trees were grown at each pH value. Detailed results have been reported (**39**), but are summarized graphically in figure 3.

EXPERIMENT VI.—In order to determine how the results obtained with the tomato in sand cultures would apply to soil conditions, a sandy loam soil was selected with a reaction of pH 4.0. Lime was added to two lots of this soil, so that three series were established; the original, pH 4.0, and the two limed series, 6.0 and 7.4. Tomato plants high in carbohydrates, with stiff woody stems and small yellowish leaves, were planted in each soil series (twenty plants at each pH) and supplied with nutrient solutions containing sodium nitrate, ammonium sulphate, and ammonium hydroxide re-

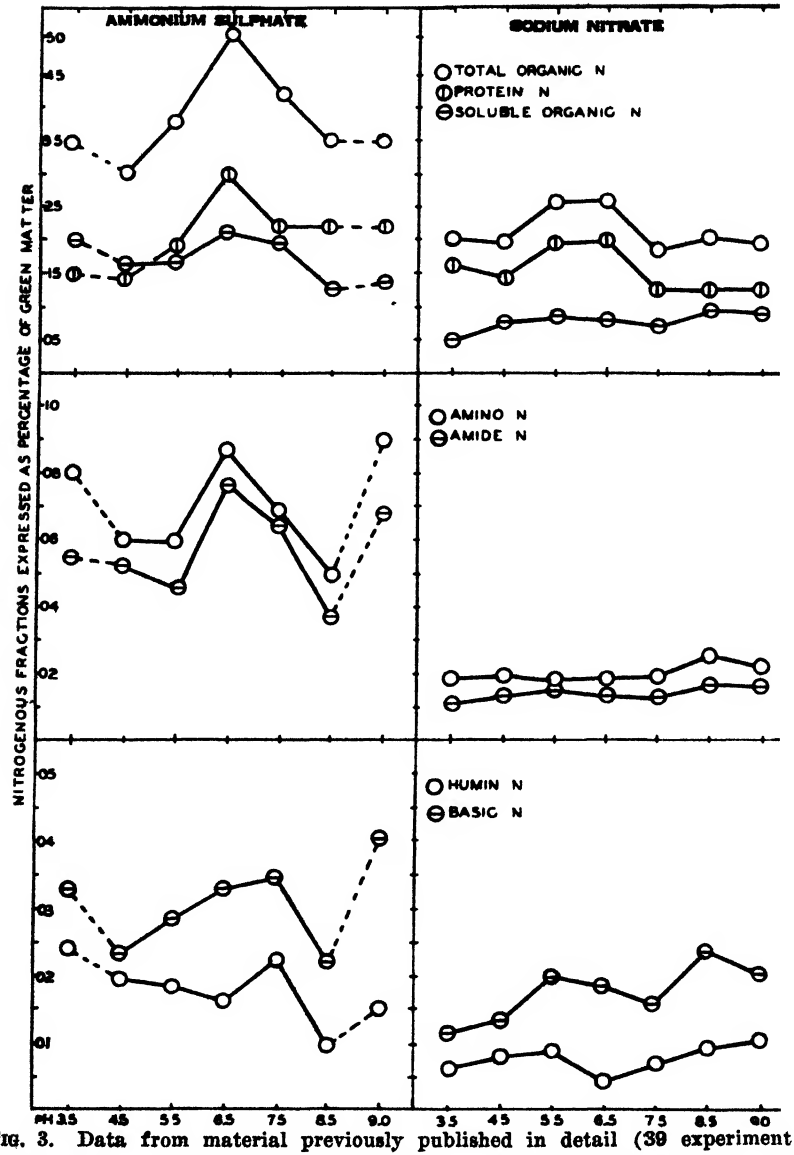


FIG. 3. Data from material previously published in detail (39 experiment III) showing relationships of H-ion concentration of nutrient medium to elaboration of nitrogenous fractions in roots of apple trees supplied with sodium nitrate or ammonium sulphate.

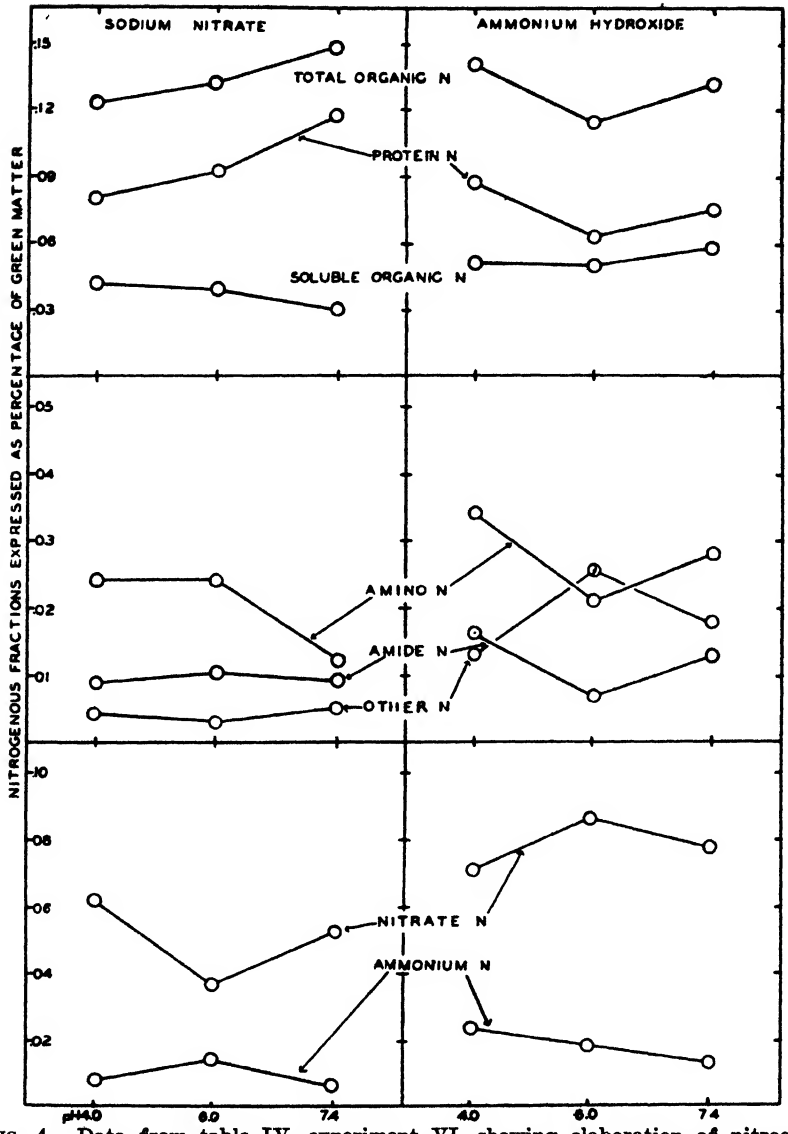


FIG. 4. Data from table IV, experiment VI, showing elaboration of nitrogenous fractions in stems of tomato plants grown on a soil adjusted to pH 4, 6.0, and 7.4 with hydrated lime and supplied with nutrient solution containing sodium nitrate or ammonium hydroxide.

TABLE IV

EXPERIMENT VI

TOMATO PLANTS GROWN IN SOIL ADJUSTED WITH HYDRATED LIME TO THE H-ION CONCENTRATION INDICATED AND SUPPLIED WITH NITRATE OF SODA OR AMMONIUM HYDROXIDE

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

Source of nitrogen	$NaNO_3$	$NaNO_3$	$NaNO_3$	NH_4OH	NH_4OH	NH_4OH	None
Dry matter (%)	6.5	6.8	6.5	5.6	6.0	6.3	7.7
pH of soil	4.0	6.0	7.4	4.0	6.0	7.4	4.0
Organic N	0.1226	0.1330	0.1473	0.1396	0.1137	0.1317	0.1214
Protein N	0.0800	0.0917	0.1193	0.0876	0.0618	0.0753	0.0671
Soluble organic N	0.0426	0.0413	0.0300	0.0520	0.0519	0.0564	0.0543
α Amino N	0.0239	0.0241	0.0125	0.0338	0.0211	0.0280	0.0324
Amide N	0.0098	0.0113	0.0093	0.0152	0.0071	0.0129	0.0235
Ammonium N	0.0082	0.0141	0.0066	0.0232	0.0178	0.0133	0.0000
Nitrate N	0.0615	0.0352	0.0525	0.0710	0.0851	0.0775	0.0000
Other N	0.0089	0.0059	0.0092	0.0130	0.0237	0.0165	0.0016

spectively. Plants when harvested (for analysis, table IV, figure 4) were 12–14 inches high; the remainder were grown to maturity. Here again the ammonium-supplied plants on the soil having a pH value of 7.4 were largest. At pH 4.0, plants supplied with ammonium sulphate made very poor growth. Microchemical tests for nitrate in the plants of the ammonium-supplied cultures showed only a trace as compared with abundant nitrates in the plants which received sodium nitrate at the same pH. Those in the cultures supplied with ammonium hydroxide at pH 4.0 did not make much growth until nitrification occurred in the soil. The plants in the nitrate cultures, in contrast to the ammonium group, made the most rapid growth at pH 4. Growth of the plants in the pH 6.0 and 7.4 cultures receiving ammonium nitrogen made a greater volume of growth than those receiving nitrate nitrogen at the same pH. The organic nitrogenous fractions are similar to those found in tomato plants grown in sand cultures with single sources of nitrogen (**40**).

EXPERIMENT VII.—During November and December, accompanied by almost continuously cloudy conditions, two series of tomato plants were grown in sand cultures at a night temperature of 58°–62° F. On occasional bright days the temperature was 5°–10° higher. One group received sodium nitrate in complete nutrient solution at an initial pH of 5.1, while the other received ammonium sulphate plus ammonium hydroxide in a complete nutrient solution at an initial pH of 8. Nutrient solutions with one-half of their concentrations made up with the nitrogenous salts (B and C, table I) were supplied to different groups of nine plants each at concentrations of approximately 1.50, 1.25, 1.00, 0.75, 0.50, and 0.25 atmospheres. Another lot of nutrient solutions (F and G, table I) were made up so that

only one-fifth of their concentration was made up by the nitrogenous salts. These nutrient solutions were supplied to different groups of nine plants each at concentrations of approximately 1.00 0.75, 0.50, and 0.25 atmospheres. Thus different groups of plants had available concentrations of nitrogen varying from extremely high to extremely low.

The plants growing with sodium nitrate made their greatest volume of growth with the highest concentration of nitrate, and the volume of growth decreased as the concentration of nitrate in the nutrient solution and in the tissue decreased. Microchemical observations showed that starch increased in the stems as the nitrate concentration decreased, but reducing sugars did not show a definite correlation with growth.

The plants of the ammonium cultures made the greatest volume of growth with the 0.50-atmosphere concentration nutrient solution containing a low concentration of ammonium salt. The two cultures receiving the higher concentrations of ammonium salt, at 1.5 and 1.25 atmospheres, made very little growth and remained grayish green, although the plants including the roots appeared uninjured. Only the stems of the ammonium-supplied plants which were growing rapidly contained starch. As the ammonium concentration in the nutrient solution increased, growth was retarded, accompanied by a deficiency of carbohydrates. Only a trace of starch could be detected in the plants supplied with the high concentration of ammonium, and that was in the endodermis. The stems of the plants tended to be hollow, and the cell walls of the xylem tissue were thinner than any of the nitrate-supplied plants.

EXPERIMENT VIII.—An experiment with Delicious apple trees similar to experiment VII with tomatoes, but conducted from January 1 to April 1, resulted in observations of a comparable nature. The trees with different concentrations of nitrate nitrogen grew fairly well (fig. 5), but growth was most rapid at one atmosphere concentration. The trees receiving the extremely low concentration of ammonium nitrogen were equal to the nitrate trees, but very little growth occurred with the high concentration of ammonium.

EXPERIMENT IX.—Tomato and cotton plants, sweet clover seedlings, and Delicious, Stayman, and Baldwin apple trees were grown in sand cultures in which the pH of the nutrient solution dripping through the sand did not increase nor decrease more than 0.2 pH from that initially supplied. Solution B (table I) was used for the nitrate cultures and solution F (table I) for the ammonium series. From 4 to 12 liters of solution, depending on the size of the plants, were dripped through sand of the individual crocks every 12 hours. Sufficient cultures were set up to give cultures from pH 3.5 to 6.5 at intervals of 0.5 pH.

FIG. 5. Delicious apple trees of experiment VIII grown in sand cultures with equivalent concentrations of nitrate and ammonium nitrogen. From left to right, culture no. 1 received a high and no. 2 a low concentration of sodium nitrate, and no. 3 a high and no. 4 a low concentration of ammonium sulphate in the nutrient solution.

The tomato and cotton plants and sweet clover seedlings assimilated ammonium most satisfactorily (made the greatest volume of green growth) at pH 6.0 and nitrate at pH 4.0. The apple varieties assimilated ammonium nitrogen most satisfactorily at pH 6.5 and nitrate at pH 4.5. The Baldwin and Stayman trees made considerable top growth and fair root growth with ammonium at pH 4.0. The Delicious trees, however, made no root growth at 4.0 and only a fair growth at pH 5.0 with ammonium nitrogen. In a later experiment it was found that if the Delicious trees were supplied with a low concentration of ammonium (0.0014 p.v.m.) the roots grew slowly although the trees did not grow. A high concentration of ammonium retarded root growth more in the Delicious than in the other two varieties.

These results are interesting because they were corroborated in the field on Rome trees growing on sandy soils of New Jersey having a reaction of pH 3.7. Calcium nitrate stimulated root growth and top growth but ammonium nitrogen did not stimulate growth. When some of this same soil

was taken into the greenhouse it was found that the trees did not respond to ammonium sulphate unless at least the equivalent of two tons of hydrated lime to the acre had been added. Sodium nitrate did not give a response unless calcium was supplied. It seemed fully as important to supply calcium in the field as in the sand cultures in order that good root growth and nitrate assimilation would take place. It was not necessary to change the reaction of the soil; however, the addition of calcium was not sufficient to cause ammonium to be assimilated. The reaction of the soil had to be increased to a higher pH value before the trees were stimulated by the ammonium. Incidentally, nitrification of the ammonium became more rapid as the pH of the soil was increased.

Discussion

The pH value of nutrient solution

Results from sand-culture experiments have a broader application to the growth of plants in their natural habitats if the pH of the culture can be maintained at a constant value. The pH value of a soil changes only slightly from year to year. Previous results on the growth of the tomato (**40**) and the apple (**39**) suggest that the pH value of the nutrient solution must be comparatively high for satisfactory assimilation of ammonium nitrogen. This may be misleading unless the pH values of the solutions, before and after dripping through the sand, are kept in mind.

When a nutrient solution containing nitrogen only as ammonium is supplied to a mature tomato plant at pH 8.0 and comes out of the culture at pH 4.0, as in the work already reported (**39, 40**), the roots of necessity are bathed in a nutrient solution of considerable variation, although the growth of the plant is very luxuriant, indicating rapid assimilation of the ammonium ion. If the initial pH of the solution is lower, growth is less luxuriant. In experiment IX it was shown that if the nutrient solution containing nitrogen only as ammonium was supplied at pH 6.0, and added in sufficient quantity to prevent a drop greater than 0.2 pH in 24 hours, growth was luxuriant. The growth at a practically constant pH of 6.0 was comparable with that produced by plants grown in a solution changing from pH 8.0 to 4.0 (**40**). This seems reasonable because the composite pH value of the variable culture was about 6.0. Similar responses to pH values were observed with cotton (**38**) and three varieties of apples (**39**). In sand culture, rapid renewal of the nutrient solution is necessary to prevent the pH from dropping materially. Results of hydrogen-ion concentration experiments previously published (**38, 39, 40**), therefore, should be interpreted on the basis of intermediate values between the initial pH of the nutrient solution and the pH after passing through the sand. The

results would then be essentially comparable with the experiments in which the nutrient solution was maintained at an approximately constant pH.

Tomato, cotton, and apple with ammonium made very slow growth at a constant pH value of 8.0, more rapid at 7.0, and most rapid at 6.0 Nutrient solutions containing nitrogen as ammonium only gave unsatisfactory results unless a constant pH of 5.0 to 6.0 was maintained. Whether trees can assimilate ammonium and make rapid growth depends on the ammonium concentration of the nutrient solution and the carbohydrate reserves in the stem. Perhaps because of the carbohydrate reserves in woody plants, ammonium may be assimilated more rapidly over a wider range of pH values than would be true of herbaceous plants. Fluctuating concentrations of ammonium are also less noticeable in the resultant growth of woody plants because of the greater supply of carbohydrates. Plants supplied with complete nutrient solutions containing nitrogen only as nitrate tended to absorb both cations and anions, so that a residual pH of 6.0 was approximated (**40**). If the nitrate was substituted by ammonium, however, the residual pH did not come to equilibrium at 6.0, but decreased to pH values as low as 3.8 (**40**). These residual changes in the nutrient solutions occurred only when plants were assimilating nitrate or ammonium nitrogen rapidly.

The absorption of ions from a solution containing nitrate apparently depends on the effect of the pH of the nutrient solution on the rate of assimilation of the nitrate or ammonium ion, or on the growth rate of the plants (**40**). The absorption of the nitrate ion decreases anions more rapidly than cations at pH 4.0, and equilibrium is maintained at approximately pH 6.0. If the pH is high enough to bring about assimilation, solutions containing ammonium nitrogen do not come to equilibrium but become increasingly more acid, owing to the residual anions and the rapidity with which ammonium is assimilated. The ammonium ion is absorbed more rapidly than the anions. This increase in acidity depends on the rate of absorption of ammonium, which in turn depends on whether the ammonium ion is assimilated as rapidly as it is absorbed from the nutrient solution. If the ammonium ion accumulates in the cell and absorption ceases, the acidity of the nutrient solution does not increase as rapidly because the residual anions do not accumulate. This may cause the residual solution to come to equilibrium at a pH value comparable with that when the nitrate ion is present. Figure 6 shows that accumulation of the ammonium ion in the plant is directly or indirectly a function of the pH of the nutrient solution. Thus it is only those plants that are assimilating ammonium rapidly which maintain a rapid absorption of ammonium ions. If the cells become saturated with ammonium ions, they can no longer absorb more.

The best growth is made with nitrate nitrogen at pH 4.0, whether the

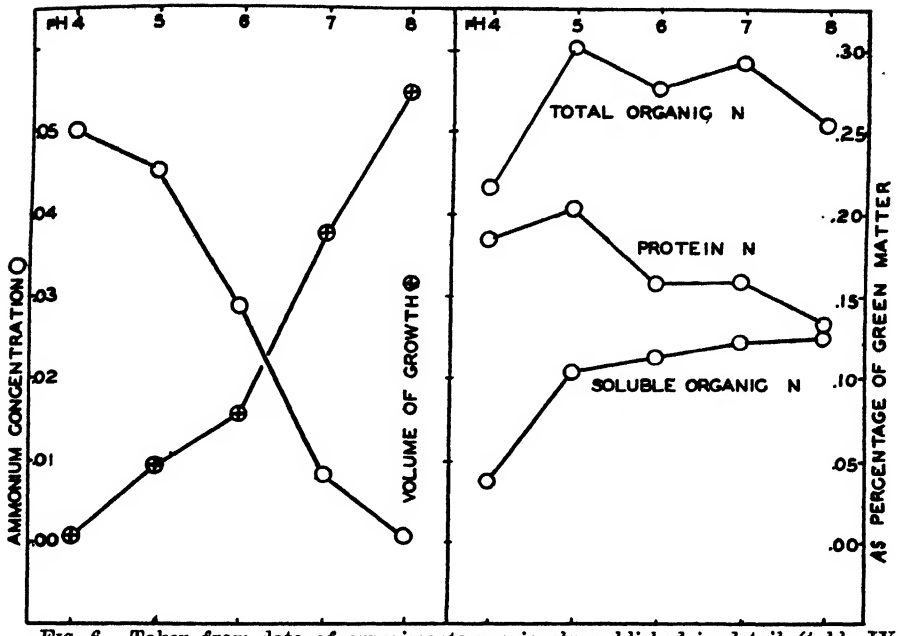


FIG. 6. Taken from data of experiments previously published in detail (table IV, 40) showing relationships between volume of growth (size of green plants in gm.) of tomato plants when supplied with nutrient solutions having different initial pH values and accumulation of ammonium in stems. Rate of assimilation of ammonia is directly correlated with growth.

pH of the residual solution remains constant or tends to come to equilibrium (**39, 40**). The pH values at which nitrate or ammonium nitrogen are satisfactorily assimilated are probably characteristic for the variety or species under observation, and are not particularly associated with the neutral point.

That plants will assimilate ammonium nitrogen satisfactorily has been demonstrated (**40**). PIRSCHLE (**29**) listed a great number of economic plants that make a rapid growth with ammonium nitrogen, and demonstrated that the pH of the nutrient solution had a controlling influence on its assimilation. He found that tobacco produced twice as much total dry matter with ammonium sulphate as with sodium nitrate at pH 6.0, although BEAUMONT *et al.* (**2**) state that tobacco does not assimilate ammonium satisfactorily. THOMAS (**36**) also states that ammonium is not satisfactorily assimilated in tobacco, and DAVIS (**7**) states that it is not assimilated in the apple; but in all instances the pH of the nutrient solutions may have been at fault, as has been demonstrated (**21, 29, 39, 40**). The pH of the nutrient solution, therefore, has directly or indirectly a controlling influence on the assimilation, particularly of the ammonium ion, and somewhat less pronounced of the nitrate ion.

Assimilation of ammonium and nitrate nitrogen

Eckerson (8) and others (25, 26) have found that plants, through the action of a nitrate-reducing material (reducase), oxidize carbohydrates or organic acids, and reduce nitrates to nitrites and ammonium, following which there are synthesized amino acids and other organic nitrogenous material. Reducase activity, however, may be affected by length of day, light intensity, or other external factors (9, 11), all of which will tend to modify the rate of assimilation of nitrate nitrogen (11). The utilization of absorbed ammonium by the tomato plants and apple trees in the experiments reported here was apparently rapid, and did not seem to be checked by lack of reducase as may possibly have been the case in the nitrate-supplied plants. This assumption would seem to be supported by the fact that amino acids increased more rapidly and in greater quantities in plants supplied with ammonium than in those supplied with nitrate nitrogen. This is shown by the data in tables II and IV for tomato and in figures 2 and 3 for apple. Likewise in 3 days the ratio of protein to non-protein nitrogen (39) in dormant apple trees changed from 73:27 to 61:39 in those supplied with nitrate, and to 56:44 in the trees of the ammonium series. Accompanying this change there was an 18 per cent. increase in total organic nitrogen in the roots of the trees receiving ammonium. Furthermore the maximum increase in total organic nitrogen in the trees supplied with ammonium occurred in 35 days, as compared with 56 days for the trees supplied with nitrate nitrogen. That a similar condition exists in the tomato is shown in table II and figure 6. Thus the general idea that ammonium is more slowly available than nitrate is not borne out by these experiments, but supports the contention (37) that ammonium may be as quickly available as nitrate nitrogen.

Eckerson (9, 10, 11) finds that some plants, including the tomato, but especially soy bean, have comparatively weak reducase activity during the short days of winter, and that carbohydrates may accumulate in the presence of abundant nitrate. It would seem, therefore, that with synthesis of organic nitrogen from ammonium there should be more rapid decrease in carbohydrates than with nitrate nitrogen under similar conditions. This is apparently borne out, at least for tomato, by figure 1 *a*, *b*. The relatively rapid decrease of starch and reducing sugars in apple roots supplied with ammonium as compared with nitrate nitrogen further emphasizes this assumption (39). Benecke (3) has suggested a similar relationship.

Observations and microchemical tests of the tomato plants of experiment VII showed definitely that during short days carbohydrates decreased as ammonium concentration increased. Nitrate-supplied plants showed con-

siderable starch, however, even at the highest concentration of nitrate nitrogen. Thus it would seem that, in order to obtain a comparable growth with ammonium and nitrate nitrogen, it is necessary to supply only as much ammonium to a culture as is equal to that made available by the reduction of nitrate in the cells of a plant growing in a nitrate supplied culture. This is supported by data presented by PIRSCHLE[3] (29). He shows that much less ammonium than nitrate nitrogen is necessary for the production of a given amount of growth.

Data by NIGHTINGALE *et al.* (27) showed that during comparatively long days in April the proportion of protein to soluble organic nitrogen was 52 to 48 per cent. in stems of highly vegetative non-fruitful tomato plants, under conditions which were favorable for both reducase (10, 11) and photosynthetic activity. This may be compared with the data on the ammonium-supplied tomato plants shown in table III, which were grown during the winter months. Here the ratio of protein to soluble organic nitrogen in the stems was 38 to 62 per cent. Thus ammonium nitrogen increased the soluble nitrogenous fraction in the tomato almost as rapidly during short winter days, when reducase activity was somewhat low, as nitrate nitrogen did under conditions favorable for abundant reducase activity. The result was a rapid utilization of carbohydrates in amino acid synthesis and extremely soft succulent growth. The stems of the tomato plants grown by NIGHTINGALE (27) contained 11–14 per cent. dry matter and were described as soft and succulent, yet plants of these experiments had only 10 per cent. of dry material in the stems. Under favorable environmental conditions, there seems to be no way to prevent extremely rapid utilization of carbohydrates in ammonium-supplied tomato plants or apple trees, except by limiting the external supply of ammonium.

Lack of carbohydrates does not favor condensation of amino acid to proteins (27, 33). It has been shown that in plants supplied with high concentrations of ammonium nitrogen (13, 39, 40) the proportion of complex protein nitrogen decreased.

The data on reducase show a paucity of activity in apple trees supplied with ammonium nitrogen (39). Apparently the synthesis of reducase did not occur, or it was inactive in the presence of appreciable quantities of ammonium. In nitrate-supplied tomato plants the rate of reducase activity seemed to prevent excessively rapid utilization of carbohydrates. In am-

[3] In the H-ion concentration studies by PIRSCHLE, it should be pointed out that many data were obtained from young plants, which have a more alkaline composite pH than mature plants. Also the concentration of ammonium nitrogen was comparatively high, so that sugars were used more rapidly than in the nitrate-supplied plants. Many of the plants which received ammonium might have synthesized as much dry matter as the plants grown with nitrate if the concentration of ammonium had been less.

monium-supplied apple trees, however, it is possible to deplete carbohydrates to the point where growth and carbohydrate storage cannot keep pace with elaboration of nitrogenous compounds. Even trees supplied with nitrate nitrogen may show signs of carbohydrate deficiency, but in a much less marked degree. This has been observed, but only during dull cloudy weather of midwinter when day length was at a minimum, whereas with ammonium nutrition it may occur in midsummer. These results are in indirect agreement with those obtained by MEVIUS and DIKUSSAR (18). They found that nitrites properly supplied were more rapidly assimilated than nitrates.

ORGANS CONCERNED IN ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN IN TOMATO AND APPLE

NIGHTINGALE (27) showed that nitrate may be found throughout the entire tomato plant. It has likewise been shown (40) that ammonium may be traced in quantity even to the leaves of the tomato plant. The data in table III show that ammonium constitutes 7.58 per cent. of the total nitrogen in the young blades and 14.46 per cent. in the old blades. Thus it seems reasonable to suppose that the assimilation of nitrate and ammonium nitrogen occurs at least to some extent in all parts of the tomato.

NIGHTINGALE (22) suggests that the fine roots of pear seedlings and blackberry bushes reduce nitrates and synthesize amino acids in the roots. KRAYBILL (14) and THOMAS (35) state that a similar condition exists in the apple. The chemical data on nitrate reduction previously presented (39), and the fact that there were no nitrates found in the stems and leaves of the apple trees, substantiate these observations. ECKERSON, however, found a trace of nitrate in the terminal buds of the 1-year-old apple trees of experiment V. The initial pH of the nutrient solution in which these trees were grown was 8 to 9, a value which was much too high for efficient assimilation of nitrate in tomato (40) and the apple (39). Nitrate may likewise be found in the tops of other plants which usually contain nitrates in the roots only, if growth conditions are unfavorable (25, 26).

An appreciable concentration of ammonium was recovered in the stems and leaves of the apple (39). This may indicate that some ammonium is assimilated in all parts of the tree. This ammonium in the upper part of the tree was probably the result of proteolysis (27), however, rather than of absorption and translocation. The distribution of ammonium is therefore no proof that ammonium assimilation and the reduction of nitrates are not chiefly root processes except in unusual cases, as has been pointed out. More ammonium was found in the woody stems and leaves of the apple (39) than in the stems and leaves of the tomato (40) when these plants were grown during the summer with ammonium nitrogen.

The data of experiment IV (39) show amino acids to be 0.132 per cent. on a green-weight basis in the stem of the ammonium-supplied trees, as compared with only 0.078 in the nitrate series. A similar comparison may be made in the leaves and both may be largely accounted for by translocation of amino acids from the roots (39). There occurred also an increase in dry matter in the roots, resulting from translocation of carbohydrates from the stems; and in view of the fact (39) that ammonium ions are almost immediately synthesized to amino acids in the presence of carbohydrates (3 days), it is probable that the larger part of the elaboration of amino acids from both ammonium and nitrate takes place in the roots. That this also is at least partly true for relatively complex organic nitrofractions is indicated by the increase in humin and "other" nitrogen.

That the nitrate ion does not go beyond the fine roots in the apple may be due to its combination with the protoplasm (ampholytes) in the root hairs. Adsorption is particularly favored when the nutrient solution has a low pH (4.5) value, and less favored in a nutrient solution at a high pH (7.5) value. This may account for the fact that ECKERSON found nitrate in the tops of trees grown in the more alkaline nutrient solution (39).

EFFECTS OF pH OF NUTRIENT MEDIUM ON ELABORATION OF NITROGENOUS FRACTIONS

In figure 3 there are recorded nitrogenous fractions in the roots of apple trees which were supplied with ammonium sulphate or sodium nitrate. These data are taken from previously published tables (39). The greater total amount of elaborated nitrogen in the ammonium as compared with the nitrate-supplied trees is evident. This is probably due to more rapid elaboration because of the greater amount of ammonium available when directly supplied than to that obtained through nitrate reduction. The greatest difference in organic nitrogen occurred when the nutrient solution of both lots of trees was supplied with an initial pH of 6.5. The greatest volume of growth of the ammonium trees, however, occurred at pH 6.5 to 8.5. This increase in total elaborated nitrogen is accounted for by a greater proportion of protein, as well as by an increase in soluble organic nitrogen. Where the volume of growth was largest (at initial pH 7.5), the protein and soluble organic nitrogen fractions are almost equal. The greatest volume of growth in the nitrate plants occurred at pH 4.5 to 5.5, whereas total elaborated nitrogen was highest at pH 5.5 to 6.5. Although the protein fractions in the ammonium and nitrate-grown trees were more or less the same in total amount, the various determined soluble nitrogenous fractions were much higher in the ammonium-supplied plants. These data are more or less in agreement with those previously presented for tomato and soy bean (40).

In the nitrate-supplied trees the effect of pH of the nutrient solution on the soluble organic nitrogen fraction of the plant is negligible except at the extreme pH values. At pH 3.5 and 9.0, at which the plants made comparatively poor growth and slight root injury was apparent, there was an increase in the soluble nitrogen fraction, possibly proteolytic (**23**). The amount of growth made by the ammonium-supplied trees follows rather generally the curves for soluble organic fractions of nitrogen, providing the data from the trees grown at pH 3.5 and 9.0 are omitted for the reasons indicated.

PRIANISCHNIKOW (**30**) emphasizes the amide nitrogen fraction in plants, and concludes that asparagine probably is a readily available reserve. He further concludes that it is elaborated when an abundance of ammonium nitrogen becomes suddenly available. MOTHES (**20**) and NIGHTINGALE (**23**), however, show that asparagine likewise increases rapidly in the dark, owing to proteolysis, and decreases in the light. This fraction is very low in the tomato (**40**), except at certain pH values, and it is probable that these exceptions in the tomato are due to "combined" ammonium (**40**) nitrogen rather than to nitrogen in the amide form. Further evidence of this will be given in a later report.

EFFECTS OF SOIL PH ON ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN IN TOMATO

Data in figure 4 show that on pH 6.0 and 7.4 sandy loam soils where ammonium nitrogen may be nitrified, the tomato absorbed rather large quantities of ammonium nitrogen, and that at these pH values the plants grew similarly to tomatoes in sand culture supplied with ammonium nitrogen at pH 7.5 to 8.5. It must be remembered that the soil cultures maintained a constant pH as compared with a variable one for most of the sand cultures. There was a greater percentage of protein at pH 4 than at 6 or 7.4, but soluble organic nitrogen increased as the pH increased. Amino nitrogen was low and amide nitrogen was high at pH 6, but was reversed at pH 7.4.

The nitrate-supplied plants made their greatest volume of growth at pH 4, which was accompanied by the largest percentage of soluble organic nitrogen in their tissues but the lowest percentage of protein. Amide nitrogen was low and amino nitrogen was high in the pH 6.0 and 7.4 cultures. Nitrate nitrogen was high in the pH 4.0 cultures, probably because of increased adsorption (**40**). The ammonium content in the nitrate-supplied plants at all pH values was low because conditions were favorable for nitrate assimilation. At pH 6 protein increased slightly in the nitrate cultures and the plants showed a slightly less succulent condition, which

was particularly emphasized at pH 7.4, where protein nitrogen was high and soluble organic nitrogen was low.

In spite of the fact that the ammonium-supplied plants were comparable in growth with plants grown in sand cultures with only ammonium nitrogen, they contained rather high fractions of nitrate nitrogen; in fact, much higher than the sand-culture plants supplied with nitrate at any pH value. The lower percentage of nitrate at pH 4.0 and high concentration of ammonium and organic nitrogen suggest nitrate assimilation. Furthermore these plants contained the least dry matter of any of the soil-grown plants, 5.6 per cent.

In the plants of the ammonium-supplied cultures at pH 6.0 and 7.4, however, ammonium was low and organic nitrogen high, as for tomato in sand cultures (**40**), indicating at these pH values efficient assimilation of ammonium and less efficient assimilation of the nitrate ion. In general the fractions of soluble organic nitrogen also follow closely in trend those previously pointed out for tomato plants in sand culture under comparable pH values of the nutrient medium. There would seem to be little doubt that the ammonium-supplied plants grown in soil produced a large part of their growth with ammonium nitrogen in spite of the absorbed nitrate nitrogen which tended to accumulate.

Summary

1. It is necessary that the limitations of the use of nitrogenous salts be determined before a fair comparison of their relative merits as plant nutrients can be made. Any of the following comparisons made between ammonium and nitrate nitrogen are on the assumption that the respective salts are supplied to the plants at the pH values of the nutrient medium or soil which are optimum for the assimilation of the particular ion under consideration.

2. The hydrogen-ion concentration of the nutrient medium directly or indirectly had a controlling influence on the assimilation, particularly of the ammonium ion. The nitrate ion was assimilated most satisfactorily in tomato and apple when absorbed from an acid nutrient solution of approximately pH 4.0. The ammonium ion was assimilated most satisfactorily when absorbed from a nutrient solution having a constant pH value of 5.0 to 6.5, varying somewhat for the variety.

3. Ammonium ions were immediately absorbed by plants without further change, and were assimilated (synthesized to amino acids and other organic nitrogenous materials) directly and more rapidly than the nitrate ion.

4. Nitrate ions were apparently absorbed, reduced to nitrite, and finally to ammonium ions by the action of reductase (nitrate-reducing material in

the plant). The ammonium ion was assimilated directly and as rapidly as it was absorbed, however, whereas the nitrate ion usually tended to accumulate, at least partly, because of limited reducase activity under certain external and internal conditions (11). The ammonium ion was, therefore, more quickly available for the synthesis of amino acids. The ammonium ion did not accumulate in plant tissue unless the plant could not assimilate this form of nitrogen.

5. The volume of growth obtained from nitrate and ammonium depended on the concentration of the nitrogenous salt in the nutrient solution and available carbohydrates.

6. There was a direct correlation between the concentration of nitrate nitrogen and the volume of growth produced if the plant was actively reducing nitrate. Plants required a much lower concentration of ammonium than nitrate nitrogen in the nutrient solution to produce an equal volume of growth.

7. Plants containing a large amount of available carbohydrates assimilated ammonium much more rapidly than those containing a comparatively small supply of available carbohydrates. Tomato and apple grown with ammonium contained a much higher concentration of soluble organic nitrogen than those supplied with an equal quantity of nitrate. There was a direct correlation between the concentration of ammonium in the nutrient solution and the amount of soluble organic nitrogen elaborated by the tomato and apple.

8. Tomato and apple growing on soil containing ammonium and nitrate ions absorbed both ions. Whether they assimilated both ions depended partly on the pH of the soil. On acid soils having a pH of 4.0, plants tended to accumulate ammonium and assimilate nitrate ions. On neutral or slightly alkaline soils, plants tended to accumulate nitrate ions and assimilate ammonium. When ammonium salts were applied to soil cultures, some of the ammonium ions were oxidized to nitric acid and were absorbed by plants as nitrate ions.

9. In general, ammonium and nitrate salts produced equally good growth, provided their limitations were recognized. Whether nitrates were reduced in the plant to ammonium nitrogen or the nitrogenous nutrient was supplied directly as ammonium, the quality of organic nitrogen in plants grown with salts from either group was similar when the total concentration of elaborated nitrogen was the same.

10. In field experiments where comparisons are made between salts of either group, comparative data may be of little value unless the condition of the soil and the quantity of nitrogenous ions absorbed and assimilated by the plants supplied with salts from either group are specifically evaluated. If salts from the two groups are used under optimum conditions for

each, the only advantage one salt would have over the other would be brought about as a secondary effect by the ion with which the nitrate or ammonium ion was associated.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

1. ADDOMS, RUTH M., and MOUNCE, F. C. Further notes on the nutrient requirements and the histology of the cranberry, with special reference to the sources of nitrogen. Plant Physiol. **7**: 643–656. 1932.
2. BEAUMONT, A. B., LARSINOS, G. J., PICKENBROCK, P., and NELSON, P. R. The assimilation of nitrogen by tobacco. Jour. Agr. Res. **43**: 559. 1931.
3. BENECKE, W. Beiträge zum Problem der Kohlensäureassimilation. Zeitschr. Bot. **13**: 417–460. 1921.
4. CHIBNALL, A. C. Investigations on the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 334–362. 1922.
5. ———. Investigations on the nitrogenous metabolism of the higher plants. III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 599–607. 1922.
6. ———. Synthesis of the i-hydroxy asparagines. Biochem. Jour. **24**: 945–953. 1930.
7. DAVIS, M. B. The influence of ammonium sulphate as a direct source of nitrogen for apple trees. Sci. Agr. **8**: 41–55. 1929.
8. ECKERSON, SOPHIA H. Protein synthesis by plants. I. Nitrate reduction. Bot. Gaz. **77**: 377–391. 1924.
9. ———. Properties of the nitrate reducing substance in plants. Amer. Jour. Bot. **16**: 851–852. 1929.
10. ———. Seasonal distribution of reducase in the various organs of an apple tree. Contributions from Boyce Thompson Institute **3**: 405–412. 1931.
11. ———. Conditions affecting nitrate reduction by plants. Contributions from Boyce Thompson Institute **4**: 119–130. 1932.
12. JONES, L. H., and SHIVE, J. W. Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on H-ion concentration and iron availability. Ann. Bot. **37**: 355–377. 1932.
13. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. **149**. 1918.

14. KRAYBILL, H. R. (A communication.) 1932.
15. LOO, TSUNG-LE. Further studies on the absorption of ammonia and nitrate by the root system of the higher plants. Bull. Dept. Biology. College of Sci., Sun Yatsen Univ., no. 10. 130 pages. (Reprint.) 1931.
16. MATTSON, SANTE. Electrodialysis of the colloidal soil material and the exchangeable bases. Jour. Agr. Res. **33**: 553–567. 1926.
17. MEVIUS, W. Die Wirkung der Ammonium Salze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. Planta **6**: 379–455. 1928.
18. ————, and DIKUSSAR, I. Nitrite als Stickstoffquellen für höhere Pflanzen. Ein Beitrag zur Frage nach der Assimilation des Nitrite stickstoffs. Jahrb. wiss. Bot. **72**: 633–703. 1931.
19. MOORE, J. C., REEVES, R. G., and HIXON, R. M. Electrodialysis as a means of studying biochemical differences in abnormal apple tissue. Plant Physiol. **2**: 313–324. 1927.
20. MOTHES, KURT. Physiologesche Untersuchungen über das Asparagin und das Arginin in Coniferen. Planta **7**: 585–649. 1928.
21. NAFTEL, J. A. F. The absorption of ammonium and nitrate nitrogen by various plants at different stages of growth. Jour. Amer. Soc. Agron. **23**: 142–158. 1931.
22. NIGHTINGALE, G. T. The growth responses of some horticultural plants and associated chemical changes in reserve foods. New Jersey Agr. Exp. Sta. Ann. Rpt. 168–170. 1927.
23. ————, ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
24. ————, ROBBINS, W. R., and SCHERMERHORN, L. G. Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. New Jersey Agr. Exp. Sta. Bull. 448. 1927.
25. ————, and ————. Some phases of nitrogen metabolism in polyanthus narcissus (*Narcissus tazetta*). New Jersey Agr. Exp. Sta. Bull. 472. 1928.
26. ————, and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
27. ————, ————, and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.

28. ————, ————, and ————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
29. PIRSCHLE, K. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. Planta **14**: 583–676. 1931.
30. PRIANISCHNIKOW, D. N. Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. deut. bot. Ges. **40**: 242–248. 1922.
31. ————. Ammoniak, Nitrate und Nitrite als Stickstoffquellen für höhere Pflanzen. Erg. d. Biol. **1**: 407–446. 1926.
32. ————, and IWANOWA, W. S. The influence of carbohydrate supply on the behavior of seedlings toward ammonium nitrate. Ann. Acad. Agron. Petroosk. 2 pp. 277–286. 1928. (Trans. title and German abst.)
33. SAPOSCHNIKOFF, W. Proteins and carbohydrates of the green leaf as products of assimilation. (In Russian.) 1894. (Abstr. in Bot. Centralbl. **63**: 246–251. 1895.)
34. THERON, J. J. Influence of reaction on interrelations between the plant and its culture medium. Univ. California Pub. Agr. Sci. **4**: 413–444. 1924.
35 THOMAS, WALTER. The seat of formation of amino acids in *Pyrus malus* L. Science **66**: 115–116. 1927.
36. THOMAS, R. P. The relation of nitrate nitrogen and nitrification to the growth of tobacco following timothy. Wisconsin Agr. Exp. Sta. Res. Bull. 105. 1930.
37. TIEDJENS, V. A. Several forms of nitrogen quickly available to trees. New Jersey State Hort. Soc. News **12**: 369. 1931.
38. ————. Growing cotton and other crop plants with ammonium nitrogen. Science **75**: 648. 1932.
39. ————, and BLAKE, M. A. Factors affecting the use of ammonium and nitrate nitrogen by the Delicious apple. New Jersey Agr. Exp. Sta. Bull. 547. 1932.
40. ————, and ROBBINS, W. R. The use of ammonia and nitrate nitrogen by certain crop plants. New Jersey Agr. Exp. Sta. Bull. 526. 1931.

CHLOROPHYLL PRODUCTION UNDER VARIOUS ENVIRONMENTAL CONDITIONS[1]

GUS B. ULVIN

(WITH EIGHT FIGURES)

Introduction

Chemical investigation of chlorophyll dates back to BERZELIUS, who, in 1838, treated the alcoholic extract of leaves with concentrated hydrochloric acid or alkali because he thought the leaf pigments were not decomposed by these reagents. He obtained only products of radical decomposition. Other investigators who used this same method were MULDER, 1844, and MOROT, 1849. It was believed by VERDEIL, 1851, that he could isolate pure chlorophyll by precipitating boiling alcoholic leaf extract with lime water and then treating the precipitate with hydrochloric acid. The hypothesis of the relationship between the coloring matter of leaves and of blood originated with VERDEIL (WILLSTÄTTER **11**).

Different theories have naturally been entertained about the composition of chlorophyll. As late as 1906 some thought that monocotyledons and dicotyledons did not contain the same kind of chlorophyll. Others were able to find several different kinds of chlorophyll in a single plant, and an unlimited number when derived from different plant sources. By far the greatest contribution to the chemistry of chlorophyll up to the present time was made by WILLSTÄTTER (**11**) and his collaborators. Their work covers the period from 1906 to 1913.

Leaf powder was used exclusively in the older methods of chlorophyll extraction and in rather large portions. Twelve-liter bottles were used to obtain "bottle extracts," and percolators, ranging up to 25 liters in size, were sometimes used for extracting chlorophyll. Loss of phytol ($C_{20}H_{39}OH$) by alcoholysis, and change in chlorophyll on long standing, are disadvantages in these longer methods.

The extraction method as suggested by SCHERTZ (**8**) in 1928 and used in this experiment makes use of fresh leaf material instead of dry leaf powder as formerly used. Grinding and extracting requires one-half hour or a little longer, in contrast with 24 to 48 hours used just for extracting by some of the older methods.

The purpose of this investigation was to study the quantitative production of chlorophyll under different light periods in soy beans and radishes, and in corn in the presence or absence of iron or manganese individually and in the presence or absence of both in the nutrient solution. It was

[1] Contribution from the Hull Botanical Laboratory, the University of Chicago.

thought possible to control the carbohydrate manufacture within the plant by controlling the light, as against controlling the nitrogen supply in the nutrient solution, which is the method usually adopted when it is desired to produce plants with either a high or a low carbohydrate-nitrogen ratio.

Methods

All of the plants used in these experiments were grown in greenhouses covered with ordinary glass. In experiment I, soy beans of the variety Manchu, and two varieties of radishes, Early Carmine Turnip and French Breakfast, were used. After two plantings of Early Carmine Turnip no more seed of that variety was available and the other was substituted. One set of plants was grown in 10-hour light periods, the other in continuous light. The short light period plants were taken out of the dark room at 8 A.M. and put back at 6 P.M. The plants receiving continuous light received the total natural daylight, supplemented by electric light during the remaining hours of each 24. Three 1000-watt Mazda bulbs with reflectors were used 26.5 inches above each group of plants to supplement daylight.

The light intensity was measured several times and it was found that approximately 25 per cent. of the full intensity of sunlight was lost to the plants in the greenhouse. On a very bright day the intensity of full sunlight was found to be 8436 foot candles, 5536 foot candles in the greenhouse, and only 2046 foot candles under a curtain of muslin used to shade the plants on bright days. On a slightly hazy day, sunlight had an intensity of 6775 foot candles, but was reduced in the greenhouse to 5239 foot candles. In the first case the loss in passing through one thickness of greenhouse glass was 34.37 per cent., and in the second, 22.67 per cent. The loss indicated by two other readings was 31.13 per cent. The intensity from three 1000-watt electric light bulbs varied from 650 foot candles at a distance of 26.5 inches from the bulbs when all were new, to 431 foot candles after several months' use.

A hundred or more soy beans were seeded in a pot of sand under natural conditions, and when six or seven days old, seven plants were transplanted into each of several 2-gallon glazed pots. These plants were grown in quartz sand with DAVIDSON'S (1) nutrient solution 3 T, R, C_8. This is a four-salt solution supplying nitrogen as nitrate nitrogen in calcium nitrate and as ammonium nitrogen in ammonium sulphate. One-half molar stock solutions were made up of each of the four salts and these added in proper amounts to distilled water to make the nutrient solution. A few parts per million of manganese and boron were added to the nutrient solution at each application and iron as ferric phosphate whenever needed. The radish plants required much more Fe than did the soy beans. The soy bean plants

were planted at intervals and each individual planting is referred to as series 1, 2, etc.

In experiment II, a garden variety of sweet corn, Golden Bantam, was used. A three-salt nutrient solution containing nitrogen only as nitrate nitrogen in calcium nitrate was used. The effect of the presence or absence of iron and manganese on chlorophyll production was studied. A series of four treatments was used in which one set of four pots received neither iron nor manganese; the second set received Fe 1 p.p.m. but no manganese; the third set received Fe and Mn at the rate of 1 p.p.m. each; and the fourth set received Fe 15 p.p.m. and Mn 1 p.p.m.

CHLOROPHYLL EXTRACTION

The method of extracting and separating chlorophyll (α and β) from the other leaf pigments in green leaves, as suggested by SCHERTZ (8), was adopted with slight modifications for this experiment. Percentage chlorophyll content of leaves on both green weight and dry weight basis as well as on an area basis was wanted. Material was often scarce, therefore 5-gram leaf samples were used instead of 10-gram samples as recommended by SCHERTZ. Five-gram leaf samples were weighed out in duplicate and traced on paper as quickly as possible. One sample was placed in a refrigerator while the other sample was being ground and extracted according to the following method:

The 5-gram sample of fresh leaves, after being weighed and traced, was placed in a glass mortar with a little sodium carbonate to neutralize free acids, and ground for seven minutes in the dry mortar. Then about 25 grams of washed quartz sand were added and the grinding continued for seven minutes, when 30 cc. of pure acetone were added and the grinding continued for another seven minutes. A total of 21 minutes of grinding for each sample was found to be the shortest time in which the leaf tissue could be thoroughly disintegrated. This acetone extract was filtered through a Büchner funnel, under suction, and the residue washed with pure acetone until the filtrate was colorless. About 100 cc. were usually required for this. The residue was then washed with about 100 cc. of ether.

The combined ether-acetone filtrate was poured into a liter separatory funnel where the acetone was washed out with distilled water. Then 100-cc. portions of water were poured down the side of the separatory funnel with the aid of a small funnel. An aqueous layer, colored somewhat yellowish from the dissolved flavones, separated out below the ether. The liquids were rotated carefully and the aqueous layer drawn off. Repeating this washing three times with each sample of material used in this experiment was sufficient to make the last washing colorless; but with old apple leaves twelve or more washings were necessary.

The remainder of the flavones and anthocyanins, if present, were removed by washing the ether solution with a 1 per cent. solution of sodium carbonate. Some of the chlorophyll may be lost by this method; but if the flavones and anthocyanins are not removed, their presence results in too high a value for the chlorophylls when estimated colorimetrically. SCHERTZ (8) found that a 1 per cent. sodium carbonate solution would remove no chlorophyll from a pure chlorophyll-ether solution. He concludes that any chlorophyll removed by this method would be chlorophyll already decomposed in the leaf.

The ether solution was run into a 500-cc. bottle and 20 cc. of a colorless saturated potassium hydroxide-methyl alcohol solution added. This was shaken strongly and set in a refrigerator until the next day.

SEPARATION OF PIGMENTS

The ether-alkali-chlorophyll solution was taken from the refrigerator and poured into a liter separatory funnel, the bottle washed several times with a few cc. of distilled water and this added to the separatory funnel. One hundred cc. of ether should be added at this point, so the bottle in which the chlorophyll solution had been stored was washed with this ether. The separatory funnel was shaken strongly and allowed to stand for 20 minutes.

The chlorophyllin layer was then run off into a small separatory funnel. The ether in the large separatory funnel was washed two or three times with small quantities of water and this added to the small separatory funnel. The ether solution was washed with a few cc. of dilute potassium hydroxide solution, and again with water to remove remaining traces of chlorophyllin salts. Both washings were added to the small separatory funnel, the contents of which were now washed with 25–50 cc. of ether. The chlorophyllin layer was run into a 100-cc. volumetric flask, made up to the mark with water, and estimated with a colorimeter in comparison with GUTHRIE'S (4) chemical standard.

CHLOROPHYLL DETERMINATION

Quantitative determinations of chlorophyll have been made by different workers but each has used a method of his own. Recently attempts at standardization of the process have been made. SCHERTZ (9) recommends Lovibond color slides as a standard. Only the depth of color was compared here because the slides used did not give the same tint as the chlorophyll solution. That same year GUTHRIE (4) developed a chemical standard to be used in colorimetric determinations of chlorophyll. This standard is a solution which contains 28.5 cc. of copper sulphate solution ($CuSO_4 \cdot 5H_2O$, 10 grams per liter), plus 50 cc. of potassium dichromate solution ($K_2Cr_2O_7$,

20 grams per liter), plus 10 cc. of twice normal ammonium hydroxide, diluted to 100 cc. This solution is colorimetrically equivalent to a solution containing 85 mg. of chlorophyll per liter. GUTHRIE'S standard was adopted because it has advantages even over a solution of the pure pigment itself; it is more stable, more easily available, and exact duplications are easily made.

Experimentation

EXPERIMENT I

EFFECT OF SHORT LIGHT PERIOD AND CONTINUOUS LIGHT ON CHLOROPHYLL PRODUCTION

Radishes from which the data were obtained were seeded on three different dates, in both short and continuous light, and harvested at different ages. The Early Carmine Turnips were seeded December 26, 1931; the French Breakfast radishes on March 31 and May 17, 1932. Only one pot of radish plants, a pot of Early Carmine, seeded December 26, 1931, blossomed in the short light period. These blossomed when 146 days old. Of those grown in continuous light the average age for four pots at blooming was 36.8 days. The midrib of the radish leaves was removed before the leaves were used for chlorophyll determinations. At first whole plants were ground up in a Russwin mill and 10-gram portions used for analysis; but this method was soon abandoned for the blades of leaves so that chlorophyll content could be determined on the area basis also.

TABLE I

AGE AND VARIETY OF RADISH PLANTS, AND PERCENTAGE OF CHLOROPHYLL IN THE LEAVES ON GREEN AND DRY WEIGHT BASIS, AND ON AREA BASIS

VARIETY	AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
		CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².
	days	%	%	*mg.*	%	%	*mg.*
Early Carmine	129	0.117	0.961	.....	.....	.....	.....
Early Carmine	132	0.134	0.690	0.040	.....	.....	.....
Early Carmine	182	0.145	1.320	0.034	.....	.....	.....
French Breakfast	33	0.068	0.861	.....	0.095	0.965	.....
French Breakfast	36	0.095	1.150	0.028	0.104	1.063	0.035
French Breakfast	40	0.107	0.970	0.024	0.097	0.51	0.026

The radish plants in the short light period did not blossom normally; the buds dropped off just before opening. In figure 1, the four plants on the right were grown in the short light period; the two in the center were

FIG. 1. Radish plants (see text for explanation).

FIG. 2. Soy bean plants 14 days old; short light period plants on left, continuous light plants on right.

FIG. 3. Soy bean plants 30 days old; short light period plants on left, continuous light plants on right.

FIG. 4. Soy bean plants 40 days old; short light period plants on left, continuous light plants on right.

FIG. 5. Soy bean plants 53 days old; same position as in figure 4.

FIG. 6. Soy bean plants all short light period: series 5, 40 days old; series 3, 53 days; series 2, 61 days old.

FIG. 7. Soy bean plants all continuous light plants: series 7, 14 days old; series 6, 30 days; series 5, 40 days; series 3, 53 days old.

FIG. 8. Soy bean plants: short light period plants on left, 80 days old; continuous light plant on right, 109 days old.

TABLE II

AGE OF RADISH PLANTS, PERCENTAGE OF CHLOROPHYLL IN LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE DIFFERENCE IN CHLOROPHYLL CONTENT

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			MORE OR LESS THAN IN SHORT PERIOD GREEN WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD PER CM².
	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².			
days	%	%	*mg.*	%	%	*mg.*	%	%	%
33	0.068	0.861		0.095	0.965		+39.70	+12.07	
36	0.095	1.150	0.028	0.104	1.063	0.035	+ 9.47	- 8.18	+25.00
40	0.107	0.970	0.024	0.097	0.510	0.026	-10.30	-47.42	+ 8.33
129	0.117	0.961							
132	0.134	0.690	0.040						
182	0.145	1.320	0.034						

182 days old, and the two on the right were 40 days old. The two on the left were grown in continuous light and are also 40 days old. The four plants on the left are in about the same stage of blossoming, but with a difference of 142 days in age. The two plants in the center, however, grown in the short light period, had reached this stage 36 days before this photograph was taken, and advanced no further; the two on the left, grown

TABLE III

AGE OF RADISH PLANTS AT BLOSSOMING IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS

VARIETY	SEEDED	SHORT LIGHT PERIOD		CONTINUOUS LIGHT	
		BLOSSOMED	AGE	BLOSSOMED	AGE
			days		*days*
Early Carmine	Dec. 26, 1931	May 20, 1932	146	Feb. 5, 1932	42
French Breakfast	Mar. 4, 1932			Apr. 8, 1932	36
French Breakfast	Mar. 4, 1932			Apr. 13, 1932	41
French Breakfast	Mar. 31, 1932			May 2, 1932	33
French Breakfast	May 17, 1932			June 19, 1932	32
Average age			146		36.8

in continuous light, had just reached this same stage in flowering. The two plants in the center were grown in the same pot in the short light period; one produced a rather large radish while the other showed no enlargement whatever. However, a majority of the radish plants grown in the short period did produce radishes, while in the continuous light enlargement seldom occurred.

The soy beans were planted at irregular intervals in both light periods, and harvested at different ages. Seven plantings were made from March 10 to May 28, 1932, and each planting is called a series; there are seven series. These plants were harvested at different ages, varying in age from 18 to 110 days. A difference can be noticed in the plants in figure 2, seven days after being transplanted and placed in the different light periods. Short period plants are on the left and continuous light plants on the right. These plants were only 14 days old. A marked contrast is noted in figure 3, in which the plants are 30 days old.

The increasing difference with age when grown in short light and continuous light periods is emphasized in figures 4 and 5, 40 and 53 days old respectively. The plants in figure 6 were grown in the shorter period, and are, reading from left to right, 40, 53, and 61 days old. In this light period the plants did not grow beyond a definite size. If vegetative growth is continued it finds expression in numerous shoots from any or all of the nodes.

The opposite condition is found in plants grown in the long light period. Up to the time of harvesting the oldest plant there was apparently an increase in vegetative vigor with age. Figure 7 shows four pots of plants at different ages but all grown in continuous light. Not all the plants in figure 8 are of one age; the seven in the pot on the left grown in the shorter period are 80 days old, and the one plant in the other pot grown in continuous light is 109 days old. New leaves and branches can be seen appearing at the nodes of the plants on the left, grown in the 10-hour light period, while the lower 13 leaves have died and fallen off the plant grown in the 24-hour period.

A marked increase in dry weight per plant in the continuous light period over the other light period was found at all ages. While the percentage increase is consistent it is not in proportion to age. Some of the environmental factors, such as temperature and humidity, that were not controlled may have been responsible for the irregularity. Surely when relative humidity approaches the saturation point with the temperature ranging from 100° to 125° F., the life processes in soy bean plants are not normal.

The ratio between roots and tops was determined for 14 different ages of soy bean plants in both light periods. Seven plants were transplanted when six or seven days old into each of the 2-gallon glazed earthenware pots. One, two, and sometimes three pots of plants were used in each light period to secure sufficient material for the chlorophyll determinations. This accounts for the different numbers of plants indicated in table IV. Table V represents the results from 195 short period plants and from 125 continuous light plants. The ratios in the long light period show a rather sudden jump to 8.50 when 32 days old, with the highest value 9.58 at 37 days. The oldest plant, 110 days old when harvested, had a ratio of 8.82, but this figure may not be reliable. Seven plants had been planted in that pot, but one soon crowded the others so badly that six of them were removed by cutting off at the level of the sand to make room for the large one. When harvested, the roots may not have been separated accurately.

In the shorter period the ratio is more irregular but they also show a wider ratio with increasing age. EATON (3) found the largest root-top ratios in the longest light periods with soy bean plants 38 days old. The same was found to be the case in this experiment up to that age, but with no increase beyond 37 days up to 110 days in continuous light. There was a drop of one-half the ratio in one instance at 42 days. This latter figure might well be disregarded. In the short light period the root-top ratio continues to increase with age and reaches 16.77 for plants 71 days old. EATON's longest light exposure was only 3.5 hours longer than the short exposure used in this experiment.

TABLE IV

AGE OF SOY BEAN PLANTS, NUMBER, TOTAL DRY WEIGHT, DRY WEIGHT PER PLANT, AND PERCENTAGE INCREASE IN DRY MATTER PER PLANT IN CONTINUOUS LIGHT OVER SHORT LIGHT PERIOD

SERIES	AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			PERCENTAGE INCREASE PER PLANT OVER SHORT PERIOD
		NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	
	days		*gm.*	*gm.*		*gm.*	*gm.*	%
6	18	21	4.09	0.194	14	4.43	0.316	62.89
4	25	21	9.29	0.442	14	7.95	0.567	28.28
7	25	21	8.27	0.393	14	10.01	0.715	81.93
2	28	14	4.55	0.325	7	5.41	0.772	137.53
5	28	14	8.34	0.595	14	15.93	1.137	89.11
3	31	9	4.70	0.522	7	12.67	1.810	246.76
5	32	14	10.60	0.757	7	10.91	1.558	105.81
4	34	7	6.10	0.871	7	10.23	1.461	67.78
4	37	7	5.39	0.770	7	20.19	2.884	274.54
6	42	14	19.91	1.422	12	33.48	2.790	96.22
1	55	7	6.70	0.957	7	26.38	3.768	293.73
5	59	18	53.21	2.956	7	66.10	9.442	219.41
3	66	14	48.44	3.460	7	74.95	10.707	209.45
2	71	14	47.85	3.42	..	....	.. ..	.....
1	110	....	.....	.	1	158.20	158.20	.....

TABLE V

AVERAGE DRY WEIGHT OF TOPS AND ROOTS OF SOY BEAN PLANTS IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS AND RATIO OF ROOTS TO TOPS

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
	TOPS	ROOTS	RATIO	TOPS	ROOTS	RATIO
	gm.	*gm.*		*gm.*	*gm.*	
18	0.144	0.050	2.88	0.216	0.100	2.16
25	0.251	0.190	1.32	0.444	0.125	3.55
25	0.334	0.059	5.66	0.586	0.128	4.57
28	0.438	0.157	2.78	1.005	0.132	7.61
28	0.271	0.054	5.01	0.678	0.094	7.21
31	0.477	0.444	1.07	1.581	0.228	6.93
32	0.642	0.114	5.63	1.394	0.164	8.50
34	0.685	0.185	3.70	1.261	0.200	6.30
37	0.740	0.107	6.91	2.598	0.271	9.58
42	1.243	0.178	6.98	2.223	0.483	4.60
55	0.857	0.100	8.57	5.490	0.600	9.15
59	2.703	0.252	10.72	8.542	0.900	9.49
66	3.202	0.271	11.81	9.642	1.064	9.06
71	3.225	0.192	16.77			
110				142.100	16.100	8.82

The age of the soy bean plants is included in tables IV, V, VI, VII, and VIII so that reference can easily be made from one table to another although not the same number of plants is included in each table. Only dry weight is used in table IV because it was thought that dry weight would be a more nearly accurate representation of the plants as a whole. Little can be said about fresh weight of roots except that some were wetter than others. That being the case the percentage of dry matter in the roots means little or nothing while the dry weight of the roots may be significant.

The largest percentage of increase per plant in continuous light over the shorter period was obtained in the plants harvested on May 4, when 55 days old. These were harvested before the temperature became abnormally high. There were no short light period plants of the same age as the oldest continuous light plant when harvested.

Chlorophyll content was determined on green weight, dry weight, and area basis. There was considerable variation in all three of these, as can be seen in table VI. On the green weight basis the leaves of the plants represented in this table usually show more chlorophyll in the long period than in the short period; 12 groups out of 13 show an increase varying from 5.41 to 53.92 per cent. The leaves showing more chlorophyll in the short light period were from plants harvested on June 19, after a few days of very hot weather, when the thermometer reached 114° F. on several

TABLE VI

AGE OF SOY BEAN PLANTS, CHLOROPHYLL CONTENT OF LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE OF DIFFERENCE IN SHORT AND CONTINUOUS LIGHT PERIOD

Age	Short light period			Continuous light			More or less than in short period green weight	More or less than in short period dry weight	More or less than in short period per cm.²
	Chlorophyll green weight basis	Chlorophyll dry weight basis	Chlorophyll per cm.²	Chlorophyll green weight basis	Chlorophyll dry weight basis	Chlorophyll per cm.²			
days	%	%	*mg.*	%	%	*mg.*	%	%	%
18	0.195	1.090	0.031	0.281	1.780	0.034	+44.10	+63.30	+ 9.67
25	0.222	1.542	0.035	0.239	1.404	0.032	+ 7.65	− 8.94	− 8.57
25	0.203	1.290	0.024	0.214	1.040	0.028	+ 5.41	−19.37	+16.66
28	0.132	0.965	.....	0.178	1.040	.....	+34.84	+ 7.77	.....
28	0.204	1.641	0.032	0.314	1.848	0.039	+53.92	+12.61	+21.87
31	0.168	1.290	0.023	0.216	1.269	0.032	+28.57	−16.27	+39.13
32	0.225	1.451	0.030	0.263	1.435	0.024	+16.88	− 1.10	−20.00
34	0.208	1.159	0.031	0.256	1.350	0.032	+23.07	+16.47	+ 3.22
37	0.225	1.180	0.037	0.256	1.340	0.035	+13.37	+13.56	− 5.40
42	0.275	1.448	0.035	0.231	1.850	0.035	−16.00	+27.76	− 0.00
55	0.238	1.192	0.034	0.256	1.340	0.037	+ 7.56	+12.41	+ 8.82
59	0.234	1.370	0.037	0.278	1.110	0.032	+18.80	−18.97	+ 1.33
66	0.234	1.064	0.035	0.279	1.111	0.035	+19.23	+ 4.41	− 0.00
71	0.266	1.210	0.040	.	. .	.....	..... .	. .....	. .....
110	.....	.... .	. .	0.230	0.838	0.033	. .....	.....	.....

TABLE VII

AGE OF SOY BEAN PLANTS, NUMBER OF LEAVES PER 5-GRAM SAMPLE, SQUARE CM. PER LEAF, AND PERCENTAGE DRY MATTER IN LEAVES GROWN IN SHORT AND CONTINUOUS LIGHT PERIODS

AGE	SHORT LIGHT PERIOD				CONTINUOUS LIGHT			
	LEAVES PER 5-GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES	LEAVES PER 5-GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES
days		*cm.²*	*cm.²*	%		*cm.²*	*cm.²*	%
18	39.00	8.00	62.40	17.85	37.00	11.08	82.00	15.70
25	35.00	9.45	66.20	14.39	26.50	13.98	74.12	17.00
25	38.00	11.10	84.40	15.71	31.50	11.86	74.75	20.60
28	24.00	13.37	64.21	12.43	16.00	25.14	80.45	17.00
31	27.50	13.11	77.29	13.00	15.00	22.11	83.19	17.00
32	24.00	15.37	73.78	15.50	15.00	30.02	90.06	18.50
34	24.50	13.72	67.24	16.66	19.50	20.35	79.40	18.84
37	21.50	14.14	60.84	17.00	10.50	34.38	72.20	19.00
42	15.50	24.82	76.96	19.00	10.75	30.58	65.74	12.50
55	19.50	14.87	58.00	20.00	5.00	68.20	68.20	19.00
59	9.75	31.97	62.35	17.00	6.12	69.54	85.19	25.00
66	13.50	24.54	66.28	22.00	5.06	77.29	78.22	25.00
71	14.00	22.67	65.29	22.00				
110					2.85	121.05	69.00	27.50
Average	23.51	16.54	68.09	17.11	15.44	41.19	79.96	19.43

occasions. During such weather the plants were watered three times a day and the floor sprinkled five or six times.

The size of the soy bean leaves varies with the age of the plants. They vary in size from 8 to 31.97 cm.² in the short light period but in continuous light from 11.08 to 121.05 cm.² In other words, the number of leaves per 5-gram sample of fresh weight varies from 39.00 to 9.75 leaves in the former and from 37 to 2.85 leaves in the latter, the larger leaves occurring on the older plants in both light periods (table VII).

In an attempt to determine the efficiency of chlorophyll in the two light periods, the ratio of the chlorophyll in the total leaves of each plant to the total dry weight of each plant respectively was calculated and these differences indicated in table VIII as greater or less than the ratios found for plants of like age in the short light period. The result was not at all striking. The ratios in the shorter period varied from 126 to 284 and in continuous light from 117 to 283. In six of the 13 comparisons the ratio was smaller in the continuous exposure; in the remaining seven the ratio was larger.

TABLE VIII

AGE OF SOY BEAN PLANTS, TOTAL DRY WEIGHT, TOTAL CHLOROPHYLL IN LEAVES, RATIO OF CHLOROPHYLL TO DRY MATTER, AND DIFFERENCE BETWEEN THIS RATIO IN SHORT LIGHT PERIOD AND CONTINUOUS LIGHT

Age	Short light period			Continuous light			Difference in ratio, above or below short light period
	Total dry weight	Total chlorophyll in leaves	Ratio chlorophyll to dry matter	Total dry weight	Total chlorophyll in leaves	Ratio chlorophyll to dry matter	
days	*gm.*	*gm.*		*gm.*	*gm.*		
18	4.09	0.0179	228	4.43	0.0337	131	−97
25	9.29	0.0515	180	7.95	0.0385	206	+26
25	8.27	0.0486	170	10.01	0.0449	223	+53
28	4.55	0.0247	184	5.41	0.0256	211	+27
28	8.34	0.0598	139	15.93	0.1346	118	−21
31	4.70	0.0337	139	12.67	0.0675	187	+48
32	10.60	0.0812	130	10.91	0.0692	157	+27
34	6.10	0.0353	172	10.23	0.0608	168	− 4
37	5.39	0.0423	126	20.19	0.1159	174	+48
42	19.91	0.1354	147	33.48	0.1411	237	+90
55	6.70	0.0358	187	26.38	0.2250	117	−70
59	53.21	0.2270	234	66.10	0.3336	198	−36
66	48.44	0.1960	247	74.95	0.3292	227	−20
71	47.85	0.1684	284				
110				158.20	0.5584	283	
Average			183			188	+5

TABLE IX

AGE OF MANCHU SOY BEAN PLANTS WHEN FIRST PODS APPEARED IN THE SHORT LIGHT PERIOD

Series	Short light period		
	Seeded (1932)	Pod appeared (1932)	Age
			days
1	Mar. 10	Apr. 20	42
2	Apr. 8	May 20	43
2	Apr. 8	May 21	44
3	Apr. 16	May 29	44
5	Apr. 29	June 11	44
Average			43.4

TABLE X

PERCENTAGE OF NITRATE NITROGEN, TOTAL NITROGEN, REDUCING AND NON-REDUCING SUGARS, STARCH, AND TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES IN STEMS AND PETIOLES OF SOY BEAN PLANTS

SOY BEAN PLANTS	NO_3 NITROGEN	TOTAL NITROGEN	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES
	%	%	%	%	%	%
Short light period, 25–28 days old	0.37	8.06	1.03	0.04	1.22	2.29
Continuous light, 25–28 days old	0.17	5.55	1.06	0.10	1.40	2.56
Short light, 42 days old	0.32	6.22	1.70	0.46	1.65	3.81
Continuous light, 42 days old	0.13	5.17	2.23	0.38	0.56	3.17

The results shown in table X seem to indicate that nitrate nitrogen was stored in the short light period plants and that the percentage of total nitrogen was also greater in these plants. Carbohydrates may have been the limiting factor in the shorter period which was caused by the limited exposure to light.

EXPERIMENT II

EFFECT OF IRON AND MANGANESE ON CHLOROPHYLL PRODUCTION IN YOUNG CORN PLANTS

Zea mays saccharata, the garden variety Golden Bantam, was used in this experiment. The plants on which chlorophyll determinations were made were harvested June 16, when 34 days old. One pot in each treatment was harvested. Sixteen days later, when it appeared that the plants were becoming crowded, two more pots in each treatment were harvested; only fresh and dry weight determinations were made on these. The gain in green weight and dry weight on the addition of both Fe and Mn, or of Fe or Mn added separately, compared with the condition when both were absent, can be considered.

Four separate treatments were used: the plants in treatment A received neither iron nor manganese; those in B received 1 p.p.m. of Fe; those in C received 1 p.p.m. of both Fe and Mn; and those in D received 15 p.p.m. Fe and 1 p.p.m. Mn in the nutrient solution applied by the drip culture method.

TABLE XI

CHLOROPHYLL PRODUCTION IN YOUNG CORN PLANTS WITH PARTS PER MILLION OF IRON AND MANGANESE IN THE NUTRIENT SOLUTION

	A	B	C	D
P.P.M. Fe	0	1	1	15
P.P.M. Mn	0	0	1	1
	gm.	*gm.*	*gm.*	*gm.*
Weight of chlorophyll in 5 grams of fresh leaves	0.0077	0.0095	0.0100	0.0127
	%	%	%	%
Percentage of chlorophyll in leaves	0.1530	0.1906	0.2000	0.2612
Percentage increase in chlorophyll over A		24.5751	30.7189	70.7189
Percentage increase in chlorophyll over B			4.9317	37.0409
Percentage increase in chlorophyll over C				30.6000
	*cm.*²	*cm.*²	*cm.*²	*cm.*²
Cm.² per 5-gram sample of fresh leaves	352.1000	371.2000	388.2000	415.8000
Chlorophyll, mg./cm.²	0.0218	0.0255	0.0257	0.0305
	%	%	%	%
Percentage of dry matter in leaves	11.5000	12.0000	14.0000	14.5000

An experiment on nitrogen assimilation by sugar-cane and sweet corn plants at different soil temperatures (**6**) was being carried on at the same time, and just prior to harvesting the sugar-cane plants, chlorophyll determinations were made on some of the leaf-blade material. These sugar-cane plants were grown in the same type of quartz sand as was used for the soy bean, radish, and corn plants. But the pots were placed in automatically controlled, electrically heated water tanks to provide soil temperatures of 15°, 25°, and 30° C. Tanks 1 and 2 were to be kept at 15° but for a time during August this temperature was exceeded. Tanks 3 and 4 were kept at a temperature of 25° throughout the experiment, and tanks 5 and 6 at 30° C. The plants in tanks 1, 3, and 5 received their nitrogen as $(NH_4)_2SO_4$ and NH_4OH, while those in tanks 2, 4, and 6 received their nitrogen as $Ca(NO_3)_2$. Both nutrient solutions had a reaction value of pH 7.

The leaves used for chlorophyll analyses were in every instance the third from the top of the plants.

TABLE XII

AVERAGE FRESH WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS WITH Fe AND Mn, GAIN IN WEIGHT PER PLANT AND PERCENTAGE OF GAIN OVER PLANTS NOT RECEIVING Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
	Number of plants	Green weight	Number of plants	Green weight	Number of plants	Green weight	Number of plants	Green weight
		gm.		*gm.*		*gm.*		*gm.*
34 days old	14	10.90	16	15.43	17	22.42	13	23.19
				%		%		%
Percentage gain over A				41.55		105.68		112.75
		gm.		*gm.*		*gm.*		*gm.*
50 days old	31	39.49	27	55.76	29	54.71	28	58.49
				%		%		%
Percentage gain over A				41.20		38.54		48.11

TABLE XIII

DRY WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS WITH Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
Age	Number of plants	Dry weight	Number of plants	Dry weight	Number of plants	Dry weight	Number of plants	Dry weight
		gm.		*gm.*		*gm.*		*gm.*
34	14	1.75	16	1.05	17	1.54	13	1.75
50	31	3.27	27	4.85	29	4.80	28	5.71
Gain in weight in 16 days		2.52		3.80		3.26		3.96
				%		%		%
Percentage gain in 16 days over A				50.79		29.36		57.14

TABLE XIV

PERCENTAGE OF CHLOROPHYLL IN SUGAR-CANE LEAVES ON GREEN AND DRY WEIGHT BASIS, CHLOROPHYLL PER SQUARE CM., AND PERCENTAGE OF DRY MATTER (6)

TANK	PERCENTAGE OF CHLOROPHYLL		MG./CM.²	DRY MATTER	PROPORTION OF CHLOROPHYLL	
	GREEN WEIGHT	DRY WEIGHT			GREEN WEIGHT BASIS	AREA BASIS
	%	%	*mg.*	%	%	%
1	0.220	0.903	0.0389	24.4	100.00	100.00
2	0.263	1.196	0.0438	22.0	119.52	112.59
3	0.223	1.016	0.0420	22.0	101.36	107.96
4	0.252	1.031	0.0500	24.4	114.54	128.53
5	0.202	0.886	0.0379	22.8	91.81	97.42
6	0.204	0.825	0.372	24.8	92.72	95.63

Discussion

The amount of chlorophyll contained in leaves necessarily limits the amount of photosynthesis under conditions as they normally exist in nature. As early as 1879, WEBER (MILLER 7) noticed that equal areas of leaves of different plants grown under similar conditions had different assimilatory powers. In 1882 HABERLANDT attempted to explain this by counting the chloroplasts in a unit area, but MILLER concludes that since there is no evidence that all chloroplasts contain the same amounts of chlorophyll no definite conclusions can be drawn from this method.

It was found by LUBIMENKO (5) that shade plants contained more chlorophyll than sun plants, and could accomplish the same amount of photosynthesis with less illumination than sun plants. He noted that if plants are grown under lowland and alpine conditions, the lowland plants may have as much as 2.3 times more chlorophyll than the alpine plants of the same species. Here there is a marked difference in the light as well as in other growth conditions. If light were the only variable factor in the environment, what differences in chlorophyll production would be found in the same species of plants?

Soy bean and radish plants were grown with this question in mind. The method adopted was to grow one set of plants in daylight from 8 A.M. to 6 P.M., supplemented by artificial light on cloudy days, and the other set in the total natural daylight supplemented as described, plus artificial light the remaining hours of the 24. During the winter season, both sets of plants received the same amount of daylight, but as the days became longer

the continuous light plants received more and more daylight in proportion to the amount of artificial light received. It would seem that this condition might be favorable to the continuous light plants.

The soy bean, a so-called short-day plant, and the radish, a long-day plant, were chosen because they were contrasting plants so far as light requirements for seed production were concerned. Under continuous light, radish plants produced flowers in from 32 to 42 days, with an average of 36.8 days. Only one pot of radishes was grown long enough in the short light period for flower production. These were planted December 26, 1931, and blossomed May 20, 1932, 146 days later. The radishes planted on the same date in continuous light blossomed February 5, 1932, when 42 days old. Temperature or light may be a factor here.

A negative correlation seems to exist between temperature and the age of radish plants at blossoming. Those seeded December 26, 1931, and grown in continuous light blossomed in 42 days, while those seeded May 17, 1932, and grown in continuous light blossomed in 32 days, a difference of more than 31 per cent. of the time required in late spring. Growth form varied considerably in both light periods, especially in the short light period. This may be noticed in figure 1.

Length of exposure to light seemed to have the opposite effect on soy bean plants. In no case were opened flowers seen on the plants, so the age used here is that at which pods were first seen in the short exposure. The range was very narrow, 41 to 44 days as compared with 32 to 42 days for radishes in continuous exposure. No pods occurred on any soy bean plant grown in continuous light although one plant was harvested when 110 days old.

The vegetative vigor of soy bean plants seemed to increase with age in continuous light. The leaves became enormous in size; only 2.85 leaves were required for 5-gram samples of fresh leaves for chlorophyll analysis. These averaged 121.05 cm.^{2} per leaf. Bean plants of series 3 were harvested in both light periods when 66 days old, and 13.50 leaves with an average area of 24.54 cm.^{2} were required for 5-gram samples of fresh leaf material in the short exposure, while only 5.06 leaves with an average area of 77.29 cm.^{2} were required in the continuous exposure. The leaves produced in continuous light have a surface area more than three times as great as those produced in the short exposure. At 32 and 18 days the figures are 24 leaves with areas of 15.37 cm.^{2}, 15 leaves with areas of 30.02 cm.^{2}, and 39 and 37 leaves with areas of 8.00 and 11.08 cm.^{2} respectively (table VII).

The thickness of the soy bean leaves was not determined, but the fourth and eighth columns of table VII indicate an appreciable difference. One gram of the fresh leaf blade material in the continuous light for 13 dupli-

cate measurements of 5 grams each averages 79.96 cm.^{2}, and in the short exposure only 68.09 cm.^{2} This average difference of 11.87 cm.^{2} of area per gram shows that the leaves were thinner in the continuous light. The leaves in the short exposure felt more leathery to the touch than the others, but the greater length and also possibly a greater number of leaf hairs in the long exposure may have accounted for that.

Only the leaves were analyzed for chlorophyll content. At an early age the plants in continuous light would naturally be expected to contain more chlorophyll than those of a corresponding age in the shorter light exposure. However, two analyses were made of 25-day old plants that had more chlorophyll in the short light period. There is considerable variation in the total chlorophyll content of the leaves as can be seen from the data of table VIII. On the green weight basis, for 13 duplicate determinations in both light periods, the average chlorophyll content was 19.83 per cent. more in continuous light; on the dry weight basis 7.20 per cent. more; and on the area basis 6.67 per cent. more than in the short exposure. Radish leaves showed an average of 12.95 per cent. more chlorophyll in continuous light on the green weight basis; 14.53 per cent. less on dry weight basis; but 16.66 per cent. more on the area basis (calculated from data of tables I and II).

An attempt was made to discover whether there is any correlation between the total dry weight and the chlorophyll content of the leaves of soy bean plants by dividing the dry weight by the amount of chlorophyll in the total leaves, assuming the remaining leaves contained the same amount of chlorophyll as the 5-gram samples used in analysis (table VIII). A high ratio would indicate greater efficiency than a lower ratio, other things being equal in the two periods; but things were not equal. There are many possibilities for errors. The greatest single error might easily be due to the fact that in the continuous exposure many of the lower leaves dropped off before analyses were made; consequently an indeterminable amount of chlorophyll was lost. No leaves dropped off the plants in short exposure, so that a more nearly accurate value for chlorophyll was obtained.

In the plants analyzed for chlorophyll when 18 days old, corresponding leaves (*i.e.*, the first two foliage leaves and one compound leaf) were gathered from each plant. Leaves from 14 plants were used in the continuous light experiments and from 21 plants in the 10-hour day experiments. The ratios of chlorophyll to dry matter in this instance are 1:228 and 1:131 in the short and continuous exposures respectively. There is an increase of chlorophyll in the total leaves in continuous light of 87.87 per cent., and the 14 plants in this set produced 8.31 per cent. more dry weight than did the other 21. But when this increase is placed on a "per plant" basis, the chlorophyll increase is 182 per cent., and the dry weight increase (table IV)

is 62.89 per cent. per plant. In view of the fact that growth conditions in each light period were maintained as nearly identical as possible, these irregularities are striking.

Cross-sections of soy bean leaves were examined under a microscope and it was thought a difference in arrangement of the chloroplasts could be noticed. The chloroplasts were more or less plano-convex, and in the continuous light these were arranged more regularly, with their flat sides toward the cell wall.

In experiment II with corn a different approach to the problem of chlorophyll production was attempted. It is conceded that chlorophyll is not formed in the absence of iron and that only very small quantities are required. The exact function of iron in chlorophyll production is not known since iron does not occur in the chlorophyll molecule. MILLER (7) cites ODDO and POLLACCI as stating that *Zea mays, Solanum nigrum, Datura stramonium, Euphorbia* sp., and *Aster sinensis* were grown in a nutrient solution in which Fe was replaced by the magnesium salt of pyrrole-carbonic acid. These investigators think that Fe has a catalytic action on the formation of the pyrrole nucleus which is the center of the chlorophyll complex. If the pyrrole nucleus is already present they think that Fe is not necessary for the formation of chlorophyll.

WILLSTÄTTER'S observations seem to support this theory, but DEUBER (2) could not confirm it. He grew corn, cow pea, soy bean, and *Spirodela* in nutrient solutions containing different proportions of a magnesium salt of pyrrole-carbonic acid substituted for iron, but in no case did this compound prevent chlorosis of the leaves of the plants.

Corn plants were grown in no. 3 quartz sand of the following composition: SiO_2, 98.95 per cent.; Al_2O_3, 0.6 per cent.; iron as Fe_2O_3, 0.31 per cent.; and no calcium nor magnesium. A nutrient solution made up of chemically pure salts was used with this sand in four different treatments as previously described. In A, the culture not receiving iron in the nutrient solution, 5-gram duplicate samples of fresh leaves were found to contain 0.1530 per cent. chlorophyll (table XI). In B, with the addition of Fe 1 p.p.m., a chlorophyll content of 0.1906 was found, a 24.75 per cent. increase over A. With the addition of Fe and Mn each 1 p.p.m., a very small increase in chlorophyll content was obtained in C; but when Fe 15 p.p.m. and Mn 1 p.p.m. were added to culture D a 30.6 per cent. increase over C was obtained. This makes a total increase of 70.71 per cent. over culture A.

The effect of Mn 1 p.p.m. with no Fe, or Mn 15 p.p.m. and Fe 1 p.p.m. in contrast to cultures B and D, was not determined; but the data indicate that iron was more effective, under the conditions of this experiment, than manganese in stimulation of chlorophyll production.

In experiment II there is a gradual increase in dry weight with the increase in chlorophyll content. The thickness of the leaves decreases with the increase in chlorophyll content per cm.^{2} The increase in dry matter, the increase in chlorophyll content on the green weight basis, and the increase in the number of cm.^{2} per gram of fresh leaf material are positively correlated in this experiment.

An increase in chlorophyll production in corn up to 67 per cent. over the check was obtained by SHULL and MITCHELL (**10**) with the use of x-ray treatments. The seeds were treated for 1, 2, 3, 4, and 5 minutes through a 1-mm. aluminum filter. The greatest increase occurred in the 2-minute treatment but all treatments showed an increase over the control.

In the experiment on the assimilation of nitrogen as NO_3 or as NH_3 by sugar-cane at soil temperatures of 15,° 25°, and 30° C., a marked difference in chlorophyll content was observed. Considering the chlorophyll content of the plants receiving nitrogen as NH_3 in the 15° C. tank as 100 per cent., the most chlorophyll was found in the NO_3 treatment at 25° C. soil temperature on the area basis. At all three soil temperatures more chlorophyll was found in the nitrate nitrogen treated plants than in those receiving their nitrogen as ammonium nitrogen. The highest percentage of chlorophyll on both the green weight and the dry weight basis occurred in the NO_3 treated plants at a soil temperature of 15° C. (table XIV).

Summary

1. Radish plants continued vegetative growth in a 10-hour day for 182 days and were still vigorous when harvested. Those in continuous light blossomed in about 36 days, soon ripened seed, and died. Radish plants in the short light period tended to store food while those in continuous light seldom produced enlarged storage organs.

2. Size of leaves in the soy bean increased with age in both light periods, but much more rapidly in continuous light. When 66 days old the leaves in continuous light were more than three times as large as those of the same age in the 10-hour period.

3. The leaves tend to be thinner with continuous light exposure, although this difference may not be significant.

4. On the average, leaves developed in continuous light contain more chlorophyll on the green weight, dry weight, and area basis than do those developed in a short light period.

5. Vegetative activity in soy beans seemed to increase with age in continuous light exposure; the reverse was the case with a short exposure.

6. Chlorophyll production was markedly stimulated in corn plants by the addition of Fe 1 p.p.m. and 15 p.p.m. to the nutrient solution.

7. Little response was obtained with Mn 1 p.p.m. Impurities in the sand may have been the limiting factor.

8. The percentage of dry matter of corn leaves increased in inverse proportion to the thickness of the leaves but in direct proportion to the chlorophyll content on green weight, dry weight, and area basis.

9. Gains in chlorophyll content are reported in corn plants as a result of x-ray treatments.

10. Under conditions of a nitrogen assimilation experiment with sugarcane, more chlorophyll was produced by the plants receiving nitrogen as NO_3 than by those receiving nitrogen as NH_3 in each of three soil temperatures.

Appreciation is hereby expressed to Drs. E. J. KRAUS and C. A. SHULL for their assistance, suggestions, and criticisms given throughout this work.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. DAVIDSON, O. W. An application of the triangle system in determining a nutrient solution suitable for research with the peach in sand culture. Proc. Amer. Soc. Hort. Sci. **25**: 354–358. 1928.
2. DEUBER, C. G. Can a pyrrole derivative be substituted for Fe in the growth of plants. Amer. Jour. Bot. **13**: 276–285. 1926.
3. EATON, S. V. Effects of variation in day-length and clipping of plants on nodule development and growth of soy bean. Bot. Gaz. **91**: 113–143. 1931.
4. GUTHRIE, J. D. A stable colorimetric standard for chlorophyll. Amer. Jour. Bot. **15**: 86–87. 1928.
5. LUBIMENKO, W. Cited by MILLER (**7**, page 465).
6. MARTIN, J. P., and ULVIN, G. B. Nitrogen assimilation as nitrate nitrogen and ammonium nitrogen by *Zea mays saccharata* and sugar-cane plants at different soil temperatures. 1932 (unpublished).
7. MILLER, EDWIN C. Plant Physiology. New York. 1931.
8. SCHERTZ, F. M. The extraction and separation of chlorophyll (α and β), carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.
9. ———. The quantitative determination of chlorophyll. Plant Physiol. **3**: 323–334. 1928.
10. SHULL, C. A., and MITCHELL, J. W. Stimulative effects of x-rays on plant growth. Plant Physiol. **8**: 287–296. 1933.
11. WILLSTÄTTER, R., and STOLL, A. Investigations on chlorophyll. SCHERTZ and MERZ, authorized translation. 1928.

RESPONSES OF KENTUCKY BLUEGRASS TO VARIATIONS IN TEMPERATURE, LIGHT, CUTTING, AND FERTILIZING[1]

CARTER M. HARRISON

(WITH SEVEN FIGURES)

Of primary importance among the several environmental factors which influence the production and maintenance of turf are seasonal variations in temperature, amount and intensity of sunlight, and cutting and fertilizing practices. It has long been observed that bluegrass grows best during the cooler seasons, although the reasons for such behavior have never been fully understood.

When bluegrass is cut short and heavily watered or fertilized, especially during the hot summer months, undesirable results follow. The turf thins out, the production of new leaves ceases, and during the cooler, wetter period of fall large numbers of the plants fail to recover. In attempts to maintain a vigorous green growth, nitrogenous fertilizers are often added to such turf when actually such an addition may be harmful instead of beneficial, especially during the hot weather of summer.

With a view to determining a possible cause for the widely differing results often obtained from apparently similar practice, the following experiments were conducted in sand culture. All cultures were obtained from the vegetative propagation of one original bluegrass plant. More particularly the experiments dealt with: (a) the effect of cutting to 0.5 inch, 1 inch, and 2 inches, plants which were supplied either with a solution high in ammonium nitrogen and low in nitrate nitrogen, or one high in nitrate and low in ammonium nitrogen; (b) the type of growth produced at different seasons of the year and the effect upon such growth of varying degrees and times of defoliation; (c) the effect of cutting plants grown with a continuous nitrogen supply as contrasted with some which had had no nitrogen during a period beginning 6 weeks prior to the initial cutting and extending to the close of the experiment; (d) the type of recovery growth produced when cultures were cut back to 1 inch in height every ten days, at 60°, 80°, and 100° F., with and without a nitrogenous fertilizer. The experiments were conducted in the University of Chicago greenhouse, from the fall of 1930 to the fall of 1932.

Several recent papers contain literature reviews of some length on much the same subjects as taken up here. GRABER, NELSON, LUEKEL, and ALBERT (**2**), PIERRE and BERTRAM (**7**), LUEKEL and COLEMAN (**6**), and GRABER (**1**) have published recent papers on the food reserves of grasses in relation to

[1] Contribution from the Hull Botanical Laboratory, the University of Chicago, under a fellowship granted by the United States Golf Association, Green Section.

growth. HARRISON (3) has published a recent paper on the effect of cutting on grass development. KRAUS and KRAYBILL (5) and THOMAS (9) have fully covered the present literature on carbohydrate-nitrogen relationships.

Experimental data

EXPERIMENT I

EFFECTS OF CONTINUED CUTTING AND FERTILIZING.—An individual Kentucky bluegrass plant (*Poa pratensis*) was propagated vegetatively, split up into 4-gm. segments and planted one segment each in 2-gallon glazed pots containing white quartz sand, free of fertilizers. The plants were started April 1, 1931, and supplied with a four-salt nutrient solution, selected from the ammonium sulphate series of JONES and SHIVE (4) made up of calcium nitrate, potassium phosphate, magnesium sulphate, and ammonium sulphate in distilled water. This was supplied by the constant drip method similar to that used by ROBBINS (8), one half of the cultures receiving a solution high in nitrate and low in ammonium nitrogen ($T_3R_1C_5$) and the other half receiving a solution high in ammonium and low in nitrate nitrogen ($T_3R_5C_1$). The pH of the solution high in nitrate was approximately 4.5, while that of the solution high in ammonium was 4.8. The cultures were grown without disturbance until September 22, when five pots of each set (ammonium high and nitrate high) were taken down and the sand washed from the roots. Figure 1 gives an idea of the different types of growth of

FIG. 1. Cultures on the left were grown in the solution high in nitrate and low in ammonium nitrogen, while those on the right were grown in the solution high in ammonium nitrogen and low in nitrate nitrogen. Note the habit of growth and the far greater number of rhizomes at the left.

the two sets. Table I gives the weights of the plant parts in grams after drying.

TABLE I

DRY WEIGHT OF PLANTS SUPPLIED WITH HIGH NITRATE AND HIGH AMMONIUM SOLUTION

TYPE OF SOLUTION		CULTURE NUMBER					
		1	2	3	4	5	Av.
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
High nitrate-low ammonium	Roots	9.5	9.1	9.5	8.5	5.2	8.3
	Tops	44.5	41.2	39.0	32.5	23.5	36.1
	Rhizomes	4.3	4.1	5.7	5.0	4 0	4 6
High ammonium-low nitrate	Roots	7.5	7.3	6.5	3.7	3.5	6.3
	Tops	42.5	38.0	30.5	19.0	16.5	29.3
	Rhizomes	2.5	2.2	1.8	0.7	1.0	1.6

The plants receiving the solution high in ammonium and low in nitrate nitrogen had leaves 15 to 22 inches long, which lay flat or drooped over the sides of the pots and were very dark green; those supplied with the solution high in nitrate and low in ammonium nitrogen had leaves 8 to 12 inches long, which were lighter green in color and stood upright in the pots. There were from 300 to 500 rhizomes in the cultures supplied with the high nitrate solution, as compared with 50 to 150 in the cultures supplied with the high ammonium solution. The former were short and thick and considerably branched as compared with the latter, which were long, thin, and unbranched.

On the same date (September 22) the leaves of five cultures of each series (high NH_4 and high NO_3) were cut to 0.5, 1, and 2 inches respectively. The clippings from this initial cutting were dried and weighed. One week later the grass was again clipped back to the same heights. It was noticed that the shorter the cutting height the shorter was the new growth made after cutting. In both sets the grass cut to 0.5 inch grew approximately 4 inches, that cut to 1 inch grew 5 inches, and that cut to 2 inches grew 6 inches. The measurements were taken above the point of the original cutting in each case. After the second cutting, during a period of high greenhouse temperatures, it was observed that the grass recovered more slowly than usual. The new leaf growth would extend upward from 1 to 3 inches and then die back from the tips of the blades. It was much more noticeable in the plants supplied with ammonium nitrogen than in those supplied with the high nitrate solution. A considerable number of the above-ground buds soon failed to produce additional new leaves or to

extend those which had been cut, and upon examination they were found to be dead. Most of the plants which died soonest were in the center of the culture, which was the oldest portion. The plants started growth from the segment which was the original planting and new rhizomes extended laterally from this segment. These new rhizomes were younger than the stem from which they originated, and none of their potential leaves had extended upward. Consequently when cutting was begun, a larger proportion of the leaf area of the older portion of the plant was removed than was true in the case of the rhizomes which had just pushed above the surface of the soil or were as yet buried beneath it. The number of leaves that a given bud will produce is fixed at an early stage in its growth. The leaves in the older buds had practically all unfolded and matured, whereas in the younger rhizomes the leaves had either just begun to unfold from the bud or as yet remained underground.

The underground parts were examined to determine what was taking place under the conditions of frequent cutting, high fertilization, and high temperatures. The cultures supplied with nitrate had from 300 to 500 growing rhizome tips on September 22 before cutting was begun, and the ones supplied ammonium nitrogen had from 50 to 150. These were all below the soil line, extending vegetatively, and producing scale leaves. On examination October 15, after the two subsequent cuttings in September, it was found that every rhizome tip below the soil line, in either fertilized set and at all three cutting heights, was dead. The terminal buds of the underground rhizomes were brown and beginning to decay. The death of the tissue back from the tip depended somewhat upon the amount of green leaf tissue remaining after cutting; the more of the green tissue removed, the greater the degree of killing of the rhizome when measured back from the terminal point. The terminal bud died first and then successively the buds at the nodes back from the tip. In most cases the entire rhizome became brown and decayed. The roots on these rhizomes died and in some cultures as much as 90 per cent. of the portion above ground was killed. The younger stems toward the periphery of the culture survived. Any rhizome that had recently emerged above the soil line and was actively producing vegetative leaves also survived. As soon as these rhizomes became above-ground stems, they produced green vegetative leaves only. These new leaves were green even below the point of shortest cutting, and probably they manufactured sufficient carbohydrates to maintain the new rhizome. The older stems had a considerable number of dry leaf sheaths at their bases, however, each new leaf produced having to extend through these dead leaves before emerging into the light. Consequently at each cutting all of the green leaf tissue produced during the period since the previous cutting was removed.. Figure 2 shows how the check plants and

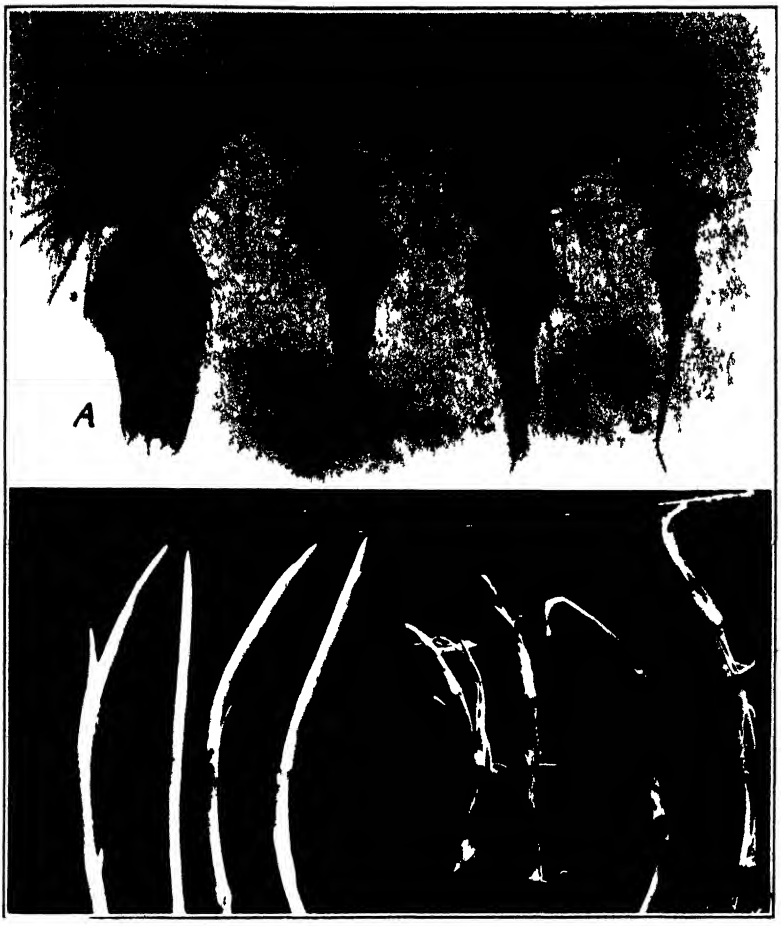


FIG. 2 Above effects of cutting treatments on bluegrass plants Plants *A* and *C* are uncut controls, plants *B* and *D* were cut down to 0 5 inch twice, the cuttings one week apart The new growth apparent on *B* and *D* is that which has appeared since the last cutting 20 days previous All the cultures were grown in sand and supplied with a nutrient solution *A* and *B* received a solution high in nitrate and low in ammonium nitrogen, while *C* and *D* received a solution high in ammonium and low in nitrate nitrogen

Below at the left, rhizomes from an uncut control, at the right, rhizomes from plants cut back to 0 5 inch in height twice, the cuttings one week apart. Photographed 20 days after second cutting

some that were cut twice appeared on October 20, approximately one month after the initial cutting The rhizomes are enlarged about two times in figure 2. It will be noted that the rhizomes from the culture not cut are white and plump with sharp terminal points, whereas those taken from the culture that had been clipped short twice are dark and shriveled.

Experiment II

Growth of bluegrass plants at different seasons.—The bluegrass plant, when started from a vegetative segment without rhizomes, began new growth by tiller-like buds in the axils of the leaves. These buds were green on emergence and grew upright almost from the start. They produced a few green scale leaves at the base but most of the leaves were large. After this initial start, short stolons which grew 1 or 2 inches from the crown of the plant before they turned upward were produced. These produced more scale leaves than the first tiller-like buds, and roots arose near the nodes. Later short rhizomes were produced. These gave rise to scale leaves underground, but upon emergence from the sand, a short way from the crown, developed vegetative leaves. This process continued until the pot was full of upright stems. Then, when the days were long and bright, the active production of above-ground stems was somewhat retarded and there were produced below the soil line large numbers of rhizomes which did not emerge so long as the top of the plant was not cut back, or so long as the days remained long and bright.

These rhizomes produced scale leaves only, and grew in length, often around the inside of the pot and sometimes down to the bottom. In late fall and early winter, as the sunlight diminished with day length and through interspersed dark cloudy periods, few new rhizomes were produced by grass that was continually supplied with nitrogenous salts. The rhizomes which were produced below the soil level during the summer and early fall, and which produced scale leaves only, gradually turned upward at the growing point, and vegetative leaves instead of additional scale leaves were produced; the new leaves turned yellowish green and emerged above the soil line. The development and emergence of these rhizomes continued until there were no growing tips below the soil line. On plants which were heavily fertilized with nitrogenous materials, during the dark winter period, a considerable number of these rhizomes as well as roots died, probably because of a deficit of carbohydrates, either as stored substance or because of the inability of the plant to manufacture them in quantity during the short cloudy days. During this period no new roots nor rhizomes were produced to take the place of those which died.

Following the emergence of the underground rhizomes, growth was initiated in the buds in the axils of the leaves above ground, and many short tiller-like branches were produced, much the same as when the plants started growth from the original plant segment. In other words, the 300 to 500 rhizomes that were produced below the soil line during summer and early fall, when the days were long and sunny, gradually emerged as the amount and intensity of light decreased, and the production of scale leaves

by these rhizomes ceased and vegetative leaves were produced. On the other hand, plants that were supplied with a minus-nitrogen nutrient solution after November 10 had produced a considerable number of new rhizomes by February 1. These increased somewhat in diameter and remained below the soil line. The tops of these plants, when the days were short and cloudy, turned yellow and grew little if any, while the root system grew deeper and more extensive. It appeared that nitrogen added at a time when the days were short and cloudy was necessary to cause emergence above the surface of the soil of rhizomes which were produced during a period of long sunny days; and that the cultures which received no nitrogen, but which were exposed to the same short cloudy days, actually continued to develop their root systems and to produce new rhizomes, with a cessation of active extension of the top. There was some death of rhizomes and roots in the cultures which were supplied continuously with nitrogenous materials. The plants supplied the minus-nitrogen solution appeared to be at a standstill both below as well as above ground, after about eight months of the treatment; and as the days became long and bright nitrogen probably became the limiting factor in growth. On the other hand, lack of sufficient light for carbohydrate synthesis in plants heavily fertilized with nitrogen probably was the limiting factor during the winter months. The difference in growth response exhibited by plants grown with and without nitrogen during the winter months is of considerable interest. The cutting factor was then introduced to see what added effect it would have on the behavior of the plants. It was found that the cutting of a plant grown under winter light conditions and supplied with nitrogen was much more disastrous than cutting one that had had no nitrogen but which had had additional light artificially added for 14 hours per day after November 10. The plants were cut back to approximately 1 inch. Table II shows how the production of top growth compared.

TABLE II

WEIGHTS OF THE CLIPPINGS PRODUCED BY TWO DIFFERENT CULTURES, ONE HAVING ADDED NITROGEN AND GREENHOUSE LIGHT ONLY, AND THE OTHER HAVING ADDED ARTIFICIAL LIGHT BUT NO ADDED NITROGEN

CULTURAL CONDITIONS	DECEMBER 15		DECEMBER 23	JANUARY 6
	INITIAL CUT GREEN WEIGHT	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT
	gm.	*gm.*	*gm.*	*gm.*
− N, + added artificial light	173	46.7	2.8	1.4
+ N, greenhouse light only	176	42.0	1.3	0.3

The culture grown with nitrogen and greenhouse light only was about 90 per cent. dead at the end of the three cuttings. When cutting was begun it had only a very few slender rhizomes. Of these, first the tips and finally the whole rhizomes died. When tips were cut from these rhizomes none of the buds near the nodes back from the cut end started growth, and in most cases the entire rhizome died. A considerable number of roots also died. Ten days after the last of the three cuttings, when the plant was lifted from the pot, only 3 or 4 inches of sand adhered to the roots. At the beginning of the test, all the sand in the 10-inch pots came out as a compact mass, held together by many fine roots.

In contrast to the culture receiving added nitrogen and natural light only, that grown without nitrogen and with added artificial light was growing vigorously after the last of three cuttings. The new growth produced, after cutting off the old yellow leaves, was dark green and became more succulent with each cutting. The large number of stocky rhizomes below the soil line, present when cutting was begun, stopped producing scale leaves and grew vegetative leaves which turned upward and finally emerged above the soil line. When tips were cut from the rhizomes in this culture, buds near the nodes back from the cut end started to grow. The first bud from the cut end elongated about 0.5 inch before dying; the second, about 1 inch; but the third one elongated sufficiently to emerge above the soil line, produce vegetative leaves, and survive. At the end of the test the roots permeated the soil mass of the entire pot, making it possible to empty the pot by lifting the plant out.

As a result of giving artificial light during the winter and withholding the supply of nitrogen, the plants if left uncut turned yellow, the leaves became stiff and upright, and very few new stems were produced above ground while a considerable number were produced as rhizomes below the soil line. When given added artificial light in the winter and a continuous nitrogen supply, the plants remained succulent and green, the axillary buds elongated above ground, produced green leaves, and there was very little if any production of underground stems. As soon as the days became longer and brighter, in March and April, the plants supplied with nitrogen produced not only a greater amount of top growth than those receiving no nitrogen but also more new roots and rhizomes.

Plants that were left to grow for a period without cutting with a continuous nitrogen supply and then cut back short appeared to undergo a reduction in the amount of underground parts, namely, the roots and rhizomes. There seemed to be a balancing of the underground portion in relation to the amount of top remaining. Then, if cutting was suspended, these plants began to produce new top growth, rhizomes, and roots much sooner than a plant that was left uncut; and in two months of long, sunny

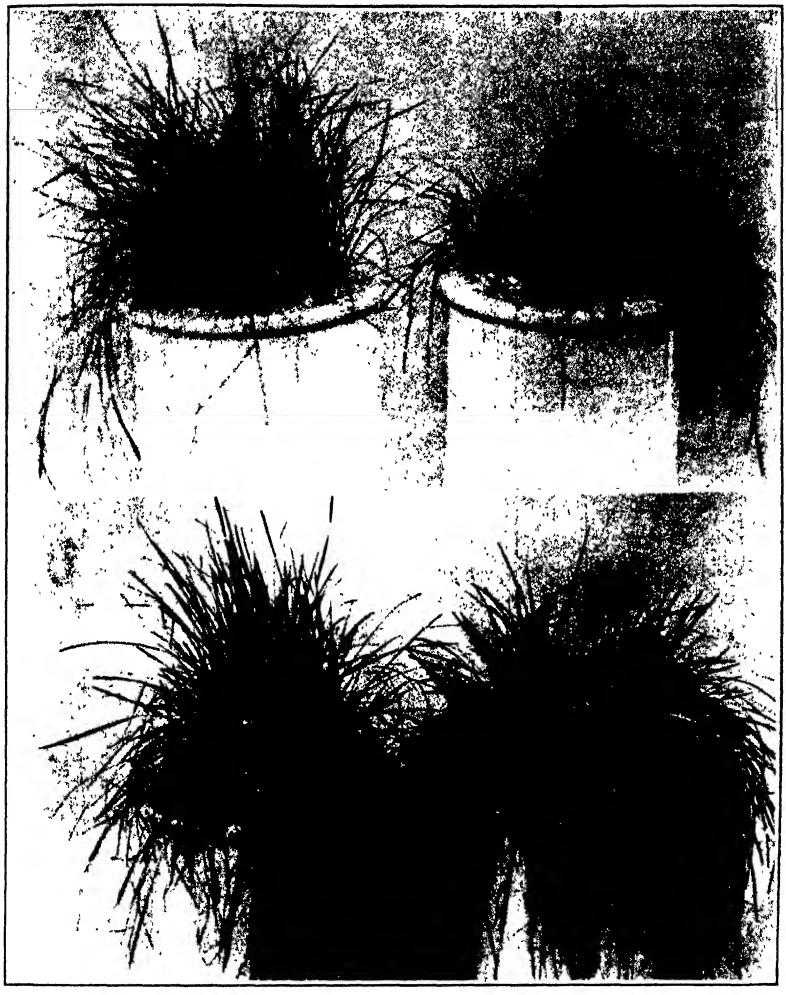


FIG. 3. Below: on the left a culture which had no nitrogen added in the nutrient solution but which had additional light after November 10 as compared with the one on the right which had a continual supply of nitrogen but no additional light. Photographed December 15; previous treatment identical.

Above: the same cultures 2 weeks after the last of three cuttings on December 15, 28, and January 6. Plants were cut back to approximately 1 inch.

days with cool temperatures the plants which had been cut and those left uncut appeared almost identical.

In order to note the amount of growth made by grass at different times of the year, cutting was continued on several of the cultures that were cut for the first time on September 22. Some were supplied with nitrogen continuously and some were given a solution containing no nitrogen. Table

TABLE III

DRY WEIGHT OF CLIPPINGS REMOVED FROM CULTURES WITH AND WITHOUT A CONTINUOUS SUPPLY OF NITROGEN AT VARYING INTERVALS OVER A PERIOD OF NINE MONTHS

CULTURE	CUT BACK TO	INITIAL CUT	SUCCESSIVE CLIPPING DATES						
		9/22/31	9/30/31	11/12/31	11/23/31	12/4/31	1/11/32	3/18/32	7/6/32
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$+NO_3$	0.5 inch	29.0	1.32	5.0	0.4	0.4	0.1	3.1	42.6
	1 "	25.0	1.05	5.7	0.9	1.0	0.4	6.9	42.5
	2 "	23.0	1.05	6.7	0.8	0.9	0,2	8.1	44.7
$-NO_3$	0.5 "	29.0	1.20	3.55	0.33	0.15	0.02	Dead	
	1 "	40.0	1.95	4.50	0.35	0.25	0.15	1.0	4.1
	2 "	34.0	1.20	6.70	0.70	0.70	0.20	0.9	3.6
$+NH_4$	0.5 "	25.0	0.50	0.10	0.02	0.03	0.02	1.1	6.5
	1 "	25.0	0.70	0.90	0.15	0.23	0.08	2.7	9.7
	2 "	29.0	1.05	1.95	0.20	0.20	0.05	2.7	6.3
$-NH_4$	0.5 "	36.0	0.60	0.50	0.07	0.15	0.05	0.6	1.6
	1 "	37.0	0.40	0.60	0.10	0.20	0.10	0.7	1.2
	2 "	27.0	0.70	1.70	0.20	0.30	0.15	1.2	1.8

III shows the weights of the clippings removed from these cultures at different times during a period of approximately nine months. The data indicate that the addition of nitrogen when the days were short and cloudy had little effect on the amount of new top growth produced.

Experiment III

Effect of cutting plants with and without an added nitrogen supply during the summer months.—The effects of cutting two cultures with different treatments, one grown with a continuous supply of nitrogen and the other supplied a nutrient solution without nitrogen, during the winter months characterized by short, cool, cloudy days, have been detailed. Tests were then made on plants during summer conditions of long sunny days with high temperatures. The plants were started on November 11, 1931, from 6-gm. segments of the same original plant used in all the previous experiments. They were supplied with a complete nutrient solution containing nitrogen in the nitrate form only. On April 21, the sand in eight cultures was washed free of nitrates and then supplied with a minus-nitrogen solution by substituting calcium chloride for calcium nitrate. The regular complete solution containing nitrate was continued on eight other cultures.

It took approximately six weeks for the accumulated nitrates in the leaves of the plants, now receiving no nitrogen, to disappear. On June 20, two months after the minus-nitrogen treatment was started, three cultures which had been receiving nitrogen and three which had received no nitrogen were dug up, the sand washed from the roots, and the plants separated into roots, rhizomes, and tops. The dry weights of the plant parts are shown in table IV. The three plants were used to serve as checks one upon the other.

TABLE IV

Dry weight of roots, tops, and rhizomes from plants grown with and without nitrogen

Cultural conditions	No.	Roots	Tops	Rhizomes	Totals
		gm.	*gm.*	*gm.*	*gm.*
Plants grown without nitrogen for two months	1	16.3	41.7	20.6	78.6
	2	16.4	38.5	21.0	75.9
	3	20.1	46.5	27.6	94.2
Plants grown with nitrogen continuously	1	13.7	59.5	15.0	88.2
	2	14.0	64.7	13.2	91.9
	3	11.8	53.2	11.7	76.7

The average dry weight of an entire plant in the minus-nitrogen set was 82.9 gm., while that in the plus-nitrogen set was 85.6 gm. The dif-

ference in dry weight between the whole plants in the two treatments was very small, but the relative amounts of the different parts of the plants, roots, tops, and rhizomes, showed considerable variation. The usual preponderance of tops to roots in the cultures supplied continuously with nitrogen is shown.

On June 21, the remaining five of each set were cut back to 0.75 inch above the level of the sand in the pot. The cutting was continued once each week thereafter, the clippings collected, and the green and dry weights recorded. Table V shows the weights of clippings removed.

TABLE V

GREEN AND DRY WEIGHTS OF CLIPPINGS REMOVED FROM CULTURES WITH AND WITH-

Conditions	No.	June 21		June 30		July 7		July 14		July 21		July 28	
		G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Cultures receiving continuous nitrogen supply	1	240	62.8	12.4	1.6	7.8	1.2	2.5	0.6	1.1	0.3	0.5	0.15
	2	192	49.2	10.1	1.5	5.8	0.9	2.6	0.6	2.8	0.8	1.3	0.4
	3	245	65.5	10.0	1.5	6.6	1.0	3.0	0.8	2.8	0.8	1.0	0.3
	4	235	61.4	10.6	1.6	8.6	1.4	2.8	0.7	1.9	0.6	1.4	0.5
	5	242	63.5	7.5	0.9	5.3	0.9	2.7	0.7	1.2	0.4	0.7	0.2
Cultures grown without nitrogen since April 21	1	121	38.2	5.1	0.7	3.2	0.6	2.9	0.9	1.5	0.5	0.9	0.3
	2	165	48.1	6.1	0.9	3.7	0.7	2.5	0.8	2.2	0.7	1.4	0.4
	3	153	47.7	5.1	0.7	3.5	0.6	1.6	0.5	2.4	0.7	1.4	0.4
	4	151	42.9	4.6	0.6	3.8	0.7	1.4	0.4	1.9	0.6	1.1	0.3
	5	173	53.9	5.5	0.8	4.5	0.8	1.8	0.5	3.9	1.1	2.0	0.6

It will be noted from table V that the cultures which received a continuous nitrogen supply produced considerably more tops, removed in the initial cutting, than those supplied with a minus-nitrogen solution. Also the clippings removed from these cultures each week, for the first three weeks after the initial cut, were greater in weight than the clippings from those receiving no nitrogen. On the average, however, those removed at the fourth cutting were greater in weight from the cultures receiving no nitrogen than from those which had had a continuous supply. For the following nine weeks this same balance in favor of the cultures receiving no nitrogen held true. The cultures supplied with nitrogen exhibited a very weak growth which was pale green in color. Those which had been growing without nitrogen started very slowly the first week after the initial cutting. They grew about 1.5 inches while the cultures supplied continuously with nitrogen grew 4 inches. The cultures without nitrogen changed in color from a yellowish green at the start of the test to a dark green which

was maintained until the close of the experiment. A considerable number of new stems appeared above ground in these cultures, while in those supplied nitrogen no new stem tips were visible.

A study of the underground parts of the cultures on June 30 showed no difference between the two sets of cultures; but on July 7, approximately two weeks after the initial cutting, a large number of the rhizome tips in the cultures supplied continuously with nitrogen were dead. In the cultures supplied with the solution containing no nitrogen there were no dead rhizome tips observable, but the growing points of the rhizomes were turning upward, and a few were producing, in place of scale leaves, yellowish vegetative leaves which were emerging above the soil line. At no time did any new rhizomes or stem tips appear above ground in the cultures supplied continuously with nitrogen. On July 20, after four weekly cuttings (that is, one month after the initial cuttings), all of the rhizome tips and a considerable number of the whole rhizomes in the cultures supplied with nitrogen were dead. No dead rhizomes were found in the cultures that had had no nitrogen since April 21. A large number of the rhizomes in these cultures had emerged above the soil line and were actively producing vegetative leaves. Some of them branched somewhat underground, but on August 15 practically all that were below ground at the start of the test had emerged above the soil line. No dead stems were noticeable in these cultures. At the same date, in the cultures which were supplied with nitrogen continuously, practically all of the rhizomes had died before emergence; likewise the roots attached to them and about 60 per cent. of the above-ground stems present at the beginning of the test had died.

TABLE V

OUT A CONTINUOUS NITROGEN SUPPLY DURING THE SPRING AND SUMMER MONTHS

August 4		August 14		August 21		August 28		September 4		September 12		September 20		September 27	
G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.	G. wt.	D. wt.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.5	0.1	0.3	0.1	0.2	0.5	0.2	0.05	Dead							
2.4	0.5	2.1	0.6	1.6	0.4	1.7	0.4	0.8	0.2	1.1	0.2	1.1	0.3	0.8	0.2
1.4	0.3	1.2	0.3	1.0	0.2	1.0	0.2	0.7	0.2	1.2	0.2	1.2	0.3	1.2	0.3
1.4	0.3	1.1	0.3	0.8	0.15	0.7	0.15	0.2	0.05	0.7	0.15	0.2	0.05	0.2	0.05
0.7	0.1	0.3	0.1	0.3	0.05	0.2	0.05	0.1	Dead						
2.2	0.5	2.1	0.7	1.7	0.5	1.7	0.4	1.1	0.4	2.4	0.5	2.2	0.6	1.4	0.5
2.1	0.5	2.7	0.8	1.9	0.6	1.8	0.4	1.2	0.4	2.2	0.5	2.2	0.6	1.2	0.4
2.3	0.5	2.7	0.8	1.9	0.6	1.6	0.4	1.1	0.4	2.2	0.5	2.1	0.6	1.5	0.5
2.1	0.4	2.2	0.6	1.6	0.4	1.4	0.35	0.9	0.2	1.3	0.3	1.2	0.3	0.8	0.2
2.2	0.5	2.6	0.7	1.8	0.5	1.6	0.4	1.2	0.3	2.5	0.5	2.5	0.7	1.5	0.5

The yield of top growth was also influenced by temperature. During the week from July 21 to 28, characterized by bright days and high temperatures, the yield decreased considerably in both sets of cultures when compared with the previous week or the following one. The day temperatures during most of the week reached a maximum of 120° F. and the night temperatures rarely went as low as 80° F. The following week (July 28 to August 4) the day temperatures reached a maximum of 90° F. and the night temperatures frequently went as low as 65° F. The week from August 27 to September 4 was also a very hot week and the yields were approximately half of what they were for the following week, which was cool.

On September 27 the experiment was discontinued. Two of the five cultures which had had a continuous supply of nitrogen were completely dead and a third was practically so. Of the two remaining, approximately 50 per cent. of the original top growth was dead. The individual plants still remaining alive were very shallow-rooted. All the rhizomes in all five cultures, together with the roots attached to them, were dead. On the other hand, the cultures which had been grown since April 21 without nitrogen had many small roots still present and alive. These cultures were beginning to show a nitrogen deficit, as evidenced by yellowing of the leaves. A considerable number of rhizome tips were still present below the soil line and no dead tips were discernible.

FIG. 4. Top row shows the five cultures which had no external nitrogen supply after April 21; bottom row shows the five cultures which had a continuous nitrogen supply. It will be noted that the two end cultures on the bottom row are completely dead.

EXPERIMENT IV

EFFECT OF CUTTING AND FERTILIZING ON GRASS GROWTH AT DIFFERENT TEMPERATURES.—It has long been recognized that Kentucky bluegrass

makes a better growth in cool weather than in hot. What effect would cutting at different temperatures have on the recovery growth of the grass? Will it grow faster at high temperatures than at low? How will rhizomes react to the different temperatures?

In order to answer these questions, some cultures which had been started on January 7 from 6-gm. segments were placed, on July 15, in constant temperature glass cases. All these cultures had received a complete nutrient solution, containing nitrogen in the nitrate form only, until May 30, after which they were supplied with a minus-nitrogen solution. The constant temperature cases were set at 60°, 80°, and 100° F. respectively. The temperatures in all of the cases varied slightly but never more than 3°. The humidity of the air was adjusted so that the saturation deficit would be approximately the same at all three temperatures. Eight cultures were placed in each case: four were supplied with a complete nutrient solution containing nitrogen in the nitrate form only, and four were supplied with a minus-nitrogen solution. The plants were left at these temperatures without cutting for five days. All of the cultures at 100° F. were beginning to turn more yellowish at the end of this period, whereas those at 80° and 60° which had been supplied with nitrogen were turning a dark green. No color change could be noted in the cultures receiving a minus-nitrogen treatment at these temperatures

On July 20 the grass was cut back to approximately 1 inch and the weights of tops removed recorded. On July 24, nine days after the beginning of the test, it was observed that the rhizomes were dying rapidly in the cultures at 100° F. They appeared to be dying faster in the cultures supplied with nitrogen than in those not receiving nitrogen. No rhizomes had died in any of the other four sets of cultures. The cultures subjected to 100° F. grew very little either with or without nitrogen. The grass was again clipped back to the 1-inch height, ten days after the initial cutting

FIG. 5. Photograph taken July 27, showing the first week's recovery growth after the initial cutting on July 20 of cultures grown at three different temperatures, with and without nitrogen.

TABLE VI

AVERAGE GREEN AND DRY WEIGHT OF FOUR CULTURES AT 100°, 80°, AND 60° F. WITH AND WITHOUT NITROGEN

DATE	100° F.−N		100° F.+N		80° F.−N		80° F.+N		60° F.−N		60° F.+N	
	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.
(Initial cutting)	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
July 20	106.6	37.9	98.5	35.9	129.0	44.8	106.4	37.5	128.2	44.2	114.1	39.7
July 30	1.05	0.29	0.9	0.25	6.5	1.5	12.8	2.5	5.4	1.1	10.6	1.8
August 9	No growth				4.0	0.9	6.4	1.2	2.4	0.47	10.4	1.8
August 20	No growth				3.0	0.8	3.7	0.8	1.6	0.4	9.8	1.8
August 31	No growth				2.8	0.6	3.2	0.5	1.6	0.3	10.5	1.9
September 12	No growth				3.5*	0.8	3.7	0.7	1.9	0.4	10.9	1.7

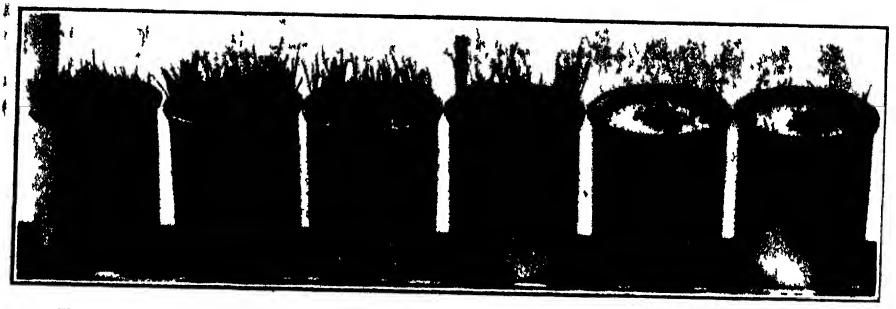
* Average of three cultures. Some solution containing nitrogen was accidentally spilled into one of the four cultures so it was discarded.

and every ten days thereafter until the close of the experiment. The weights of the tops removed from each culture are recorded in table VI.

The cultures at 100° F. were discontinued August 31 as none of them were growing and all of the original tops appeared dead with the exception of two or three leaves in the minus-nitrogen set. The pots were left in the greenhouse and watered daily. The temperature reached a minimum of around 70° F. during the night, which was considerably cooler than the temperature to which they had been previously exposed. On September 12 it was noted that a few spindling light green leaves were appearing around the edges of all the cultures except one of the four which had been supplied with nitrogen. Upon closer examination it was observed that this growth was coming from some of the dormant lateral buds on a very few of the rhizomes, in no instance from the terminal bud.

FIG. 6. On the left two rhizomes which were completely dead. The next five show the nature of the recovery growth from the rhizomes in the 100° F. cultures. Note the new growth back from the tip of the rhizome and the dead portion above it. Next to these five is shown a rhizome which had just emerged above the soil line and was producing vegetative leaves. The last two on the right show the appearance of the rhizomes underground and producing only scale leaves.

At the end of the test the cultures were separated into living tops, dead tops, roots, and rhizomes. No attempt was made to separate the dead underground tissue from the living portions. Table VII shows the weights of the different portions at the close of the test.



To replace Fig. 7, page 100 in the January number of PLANT PHYSIOLOGY.

FIG. 7. Photograph taken September 20 showing nature of the top growth produced during the last week of the test. Note the thickening up of the grass in the cultures at 60° + N and the spindling growth in the cultures exposed to 100° until August 31.

TABLE VII

DRY WEIGHT OF PLANT PARTS AT END OF TEST (AVERAGE OF FOUR CULTURES)

PARTS	100° − N	100° + N	80° − N	80° + N	60° − N	60° + N
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Roots	10.5	9.9	12.2	9.3	16.4	11.2
Rhizomes	6.1	7.0	7.4	7.7	12.9	9.2
Living tops	0.25	0.16	7.7	2.8	8.4	12.3
Dead tops	4.5	4.5	1.6	4.9	None	None

Table VII shows that the roots and rhizomes in the cultures at 60° without nitrogen weighed far more at the conclusion of the test than those of any of the other cultures; also, that there were no dead tops whereas there were many at the other two temperatures. Obviously either high temperatures or the addition of nitrogen may bring about a decrease in the weight of the underground storage portions of the plant.

It will be observed from figure 5 and table VI that the grass made very little recovery growth either with or without nitrogen at 100° F. After the second cutting these cultures did not produce any new top growth until after the exposure to 100° F. was discontinued. The tips of the rhizomes were practically all dead in both sets and very little green top growth was noticeable. At 80°, the production of new leaves after cutting was very rapid when nitrogen was added and much slower in the minus-nitrogen treatment. The leaf blades in the cultures without nitrogen were short and broad, while those receiving nitrogen were long and narrow. None of the rhizomes present at the beginning of the test had begun to appear

in either set. On closer examination, it was observed that the tips of a number of rhizomes were dying underground in the cultures receiving nitrogen, whereas those supplied with a minus-nitrogen treatment were turning upward and growing toward the surface of the sand. The weight of the clippings removed after the first ten days from the cultures receiving a minus-nitrogen treatment was approximately one-half of that removed from the cultures receiving nitrogen. One month later the clippings removed were approximately the same in both sets, with or without nitrogen. At 60°, in the cultures supplied with nitrogen, the first observable change was the emergence of a large number of rhizome tips, which were underground at the beginning of the test. These had grown rapidly upward through the sand and emerged into the light. Tiller-like buds at the base of the leaves of the older above-ground stems were also beginning to grow and produce vegetative leaves. The top growth of these cultures, which consisted primarily of upright leaf blades, became considerably denser, and although the grass did not grow so tall as it did at 80° with nitrogen, the weight of the recovery growth was almost equal. One month after the initial cutting, the figures show that the cultures supplied nitrogen at 60° were producing approximately three times as much weight of tops as the cultures receiving nitrogen at 80°. This difference was largely due to the increased number of new, above-ground stems in the cultures at 60° which were producing leaves. At 60°, when the cultures were supplied with nitrogen the rhizomes grew upward into the light, while at 80° they died before emerging and no new tiller-like buds were produced by the older above-ground stems. Much more green leaf tissue was left after cutting in the case of the grass in the cultures at 60° supplied with nitrogen than at 80°, because these new stems were green on emergence and had considerable leaf area left below the cutting point; whereas most of the green tissue was removed from the cultures at 80°. The cultures at 60° without nitrogen very slowly produced new top growth following cutting, and upon closer examination it was noted that these plants were actively producing new roots, a characteristic not observable in any of the other sets of cultures. Even after the fourth successive cutting these cultures were still producing new roots.

Summing up the general observations on the effects of the variations in temperature, the following points can be noted: (1) at 60°, minus nitrogen, the top growth recovery following cutting was slow, none of the rhizomes pushed out above the soil, and the cultures produced many new roots; (2) at 60°, plus nitrogen, no new roots were produced but many of the rhizomes below ground at the beginning of the test turned upward and pushed above the soil line; (3) at 80°, minus nitrogen, no new roots were produced and some of the rhizomes gradually appeared above ground; (4) at 80°, plus

nitrogen, no new roots were produced, no rhizomes appeared above ground, and a large number of rhizomes and roots died; (5) as the number of cuttings increased, the yield of tops between cutting periods in the cultures receiving the treatment of minus-nitrogen at 80° was beginning to approximate the yield of those receiving nitrogen; (6) at 100°, in either set there was very little new top growth and the rhizome tips and the roots practically all died.

A final consideration of the results of the various experiments, as set forth in the foregoing tables and illustrations, brings out several characteristic responses of Kentucky bluegrass to changes in temperature, amount and intensity of light, variations in nitrogen supply, and cutting practices.

Tables II and III and figure 3 show that bluegrass did not grow the same in winter when the days were short and cloudy as it did in summer when the days were long and sunny. The plants produced the largest amount of new tops, roots, and rhizomes during the spring and fall when the temperatures were not excessively hot or cold, and the days were bright and long. The plants, during the long, sunny days of spring, summer, and fall, produced a large number of rhizomes if continuously fertilized with nitrogen and left uncut; but as the short cloudy days of winter arrived, these rhizomes turned upward and emerged into the light above the soil line. If the nitrogen supply was discontinued during this period of short, cloudy days, the rhizomes not only remained below the level of the ground but became stockier and increased in number. Severe cutting of the tops of a culture continuously supplied with nitrogen was much more disastrous during this short cloudy period than during the period of long, bright days of spring, early summer, and early fall. If the days were long and sunny but characterized by excessively high temperatures, the cultures produced less new growth between cuttings than they did when the temperatures were lower.

On the other hand, close and frequent cutting of a plant that had been growing for some time without an external supply of nitrogen and the leaves of which had turned from green to yellow did not bring about the death of any of the above-ground stems, roots, or rhizomes. This plant had a large number of thick, tough rhizomes; the plants continuously supplied with nitrogen and grown during the period of long sunny days had fewer and more succulent rhizomes; whereas the cultures grown with a continuous supply of nitrogen during the winter had none. The rhizomes of the latter had either come up to the surface or died before emergence. It seems evident that the rhizomes may store a supply of carbohydrates which may be used by the plant during periods of short and frequent clipping or during periods of dark, cloudy weather. When the plants were kept extremely vegetative very few rhizomes were formed, and when in this

condition, close and frequent defoliation resulted not only in lessened production of new leaves but actually caused death of the stems above ground, and of roots and rhizomes.

The plants which were grown in the winter period of dull, cloudy days, and which received a continuous supply of nitrogen, had practically no rhizomes and therefore had very little underground storage supply of carbohydrates. If the tops of these cultures were kept cut off continuously the plants died very soon, while others without nitrogen and which had a large number of thick, sturdy rhizomes continued to produce new top growth; and none of the roots, rhizomes, or above-ground stems died during the course of the experiment. As the cutting continued, the rhizomes of the plant without nitrogen gradually turned upward, and emerged above the soil line and produced mainly vegetative leaves. The amount of defoliation that a grass plant will survive is largely dependent upon the rate at which the carbohydrates, stored in the underground portions of the plant, are used up. When the plant is completely defoliated, the drain upon the reserve carbohydrates is much more severe than it is when considerable leaf area remains after cutting. When temperatures are between 70° and 90° F., and there are sufficient moisture and nutrients, the new leaves produced grow quickly and rapidly. The constant removal of this new growth soon brings about the death of these plants through carbohydrate starvation, whereas with constant cutting a plant with high carbohydrate storage and without an external supply of nitrogen grows slowly during the periods between cuttings, thus utilizing smaller amounts of the stored carbohydrates and continuing growth over a longer period of time. In the case of the vegetative plant, a large amount of top growth is removed at each cutting; but such cutting can continue only over a short period of time, after which the development of new leaves becomes slower and slower until the plant in many cases actually dies. In contrast to the vegetative plant, a small amount of top growth is removed from the non-vegetative plant at each cutting, but the production of new leaves continues over a much longer period of time.

These results as a whole indicate that fertilization of grass with nitrogenous fertilizers during periods of short and frequent cutting at high temperatures does not result in an increase of growth but may even result in a decrease. Several factors, such as short and frequent clipping, shade, short cloudy days, nitrogen fertilizers, heavy watering, and high temperatures tend toward the using up of such supply of stored carbohydrates as may be available, and if carried to such an extreme that these carbohydrates are completely utilized and are not made available through the activity of additional leaf area, ultimate death of the plant may be brought about. Short and frequent cutting and fertilizing with nitrogen may help consid-

erably in the production of a denser turf provided the plants have a carbohydrate reserve, or a ready means of its manufacture, and the weather remains cool and bright.

Summary

1. The leaf blades of bluegrass cultures supplied with calcium nitrate were shorter and more upright, the rhizomes were more numerous, stockier, and more branched than was the case when the plants were supplied with ammonium sulphate.

2. The rhizomes and a large proportion of the roots of vegetative cultures were killed by short and frequent defoliations.

3. The plants exhibited a different type of growth at different seasons of the year, when light conditions, nitrogen supply, and cutting practices were varied. Rhizomes produced in cultures left uncut during the bright cool days of spring, early summer, and early fall and continuously supplied with nitrogen, gradually emerged into the light as the short cloudy days of late fall and winter arrived. By limiting the nitrogen or increasing the light, these rhizomes not only remained below the soil level but became stockier and new ones were also produced.

4. It was possible to influence the amounts and relative proportions of various plant parts by limiting the nitrogen supply. As the nitrogen supply was decreased, the relative amount of top growth diminished as the roots and rhizomes increased.

5. Cutting back the leaves of non-vegetative plants supplied with a minus-nitrogen nutrient solution, and which had a large quantity of storage rhizomes, was less harmful during the hot summer months than was short cutting of vegetative plants which received a continuous supply of nitrogen and which had a much smaller quantity of storage rhizomes. It was possible to kill plants that were continuously fertilized with nitrogen by frequent defoliations.

6. Plants grew very little at 100° F., after defoliating, with or without nitrogen; and a large part of the established cultures died during six weeks of such exposure. A few dormant buds on the rhizomes recovered after the exposure was discontinued and the temperature lowered. In no case was such recovery from a terminal bud on the rhizome but rather from a bud several nodes back from the tip.

7. When cultures exposed to 80° F. and supplied with nitrogen were periodically defoliated, new leaves were produced which grew rapidly. This rapid growth exhausted the reserve supply of carbohydrates during the first few periods; and as the defoliations were sufficiently frequent to prevent a replenishing of the supply, the growth made between cuttings became less and less. Finally these cultures were producing no more new

growth between the periods of defoliation than were cultures exposed to the same temperature but supplied with a minus-nitrogen solution. The tips of the rhizomes in the cultures supplied with nitrogen died, while the rhizomes in those receiving no nitrogen turned slowly upward and emerged above the soil line.

8. At 60° F., when the cultures were supplied with nitrogen the new leaves produced after a defoliation grew much more slowly than was the case at 80° F., but soon practically all of the rhizomes present at the beginning of the test had emerged above the soil line, and tillered profusely on emergence; and these cultures, because of this different habit of growth, were soon producing a much higher yield of new growth between defoliations than were the cultures at 80° F.

9. At 60° F., without nitrogen, the response to close cutting was the production of many new roots. During the course of the experiment the rhizomes remained below ground and no dead tips were discernible. The cultures yielded very little new top growth during the periods between defoliations.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. GRABER, L. F. Food reserves in relation to other factors limiting the growth of grass. Plant Physiol. **6**: 43–72. 1931.
2. ————————, NELSON, N. T., LUEKEL, W. A., and ALBERT, W. B. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisconsin Agr. Exp. Sta. Bull. 80. 1927.
3. HARRISON, C. M. Effect of cutting and fertilizer applications on grass development. Plant Physiol. **6**: 669–684. 1931.
4. JONES, L. H., and SHIVE, J. W. The influence of ammonium sulphate upon plants supplied with ferric phosphate and ferrous sulphate as sources of iron. Jour. Agr. Res. **21**: 701–728. 1921.
5. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
6. LEUKEL, W. A., and COLEMAN, J. M. Growth behavior and maintenance of organic foods in Bahia grass. Florida Agr. Exp. Sta. Bull. 219. 1930.
7. PIERRE, W. H., and BERTRAM, F. E. Kudzu production with special reference to influence of frequency of cutting on yield and forma-

tion of root reserves. Jour. Amer. Soc. Agron. **21**: 1079–1101. 1929.

8. ROBBINS, W. R. Nutrient requirements of the sweet potato plant when grown in sand culture, and effect of various nutrient salt proportions on the composition of the roots. New Jersey Exp. Sta. Ann. Rpt. pp. 178–179. 1927.
9. THOMAS, WALTER. Composition of current and previous season's branch growth in relation to vegetative and reproductive responses in *Pyrus malus* L. Plant Physiol. **7**: 391–445. 1932.

HYDRATION OF SOLUTE IONS IN RELATION TO ACIDITY, ALKALINITY, AND pH

LEWIS H. FLINT

Introduction

During the last few years an increasing amount of research has been directed to the solution characteristic called "acid" or "alkali," and more recently interpreted as a concentration of H^+ ions and expressed as pH. On the one hand this attention has been accompanied and fostered by an increasing recognition of the importance of the characteristic in the precise control of varied solution reactions in industrial processes, and in its practical application to such processes as measured by colorimetric or electrometric methods, it has been widely accepted as a means of standardization and control. On the other hand the attention has given rise to divergent interpretations of the factors responsible for the characteristic. The view embodied in such treatises as those of CLARK (**9**), MICHAELIS (**18**), and BRITTON (**7**) is that the concentration of H^+ ions is responsible for the attribute. Yet GORTNER (**14**) and others have found no satisfactory assurance in this interpretation, and have substituted "ionic activity" for "hydrogen ion concentration" in explanation. The possible effectiveness of other than H^+ ions has thus been recognized.

During the last few years also an increasing divergence of opinion has developed in regard to the state of dissolved substances. Following the wide acceptance of the ionization theory of ARRHENIUS, there came a wide acceptance of the theory of incomplete dissociation, a theory developed largely in explanation of the more or less parallel decreases in specific molecular electrical conductivities and freezing-point depressions with increasing concentrations. During the régime of the wide acceptance of this theory most of our present texts were written, so that we may look upon it as the orthodox system. Yet with an increasing amount of evidence pointing to complete ionization at all concentrations, a situation has arisen in which, on the one hand, many investigators take complete ionization for granted, while on the other hand many others assume incomplete ionization. Still more recently others have sought to build on the DEBYE-HÜCKEL theory as a sort of mathematical compromise involving electrical forces.

Whatever view-point one has acquired with respect to the validity of the pH interpretation of acidity or with respect to ionization, however, it is inevitable that the increasing differences of opinion in regard to these matters further complicate the direction of research in solutions. From the study of pH measurements involving controllable amounts of known materials, we may obtain ample assurance that these measurements afford a

means of marking a definite and reproducible stage in various processes. The measurements are thus of great practical value, independent of the validity of any interpretation. From the study of pH measurements involving unknown amounts of unknown materials, however, we obtain little assurance. It appears that the same pH may characterize both a favorable and an unfavorable medium; that the pH of an organism may be modified by the medium or the pH of the medium may be modified by the organism; and that the amount of buffering material in either the organism or the medium may condition the measurement. Under these circumstances it appears that we should not unhesitatingly accept the postulate that solute ions other than H^+ ions (or H^+ and OH^- ions) have little or no influence on the pH measurement. Yet if other ions do influence the measurement, what is the manner of their action and how may we evaluate it? The subject is a precarious one, for the widely recognized usefulness of the pH measurement in marking the progress of industrial processes has fostered the acceptance of the H^+ ion interpretation; and any departure from this interpretation must meet a great deal of criticism. On the other hand, since the acceptance of the H^+ ion interpretation appears inconsistent with many observational data of physiological and physico-chemical research, we are not honest with ourselves if we do not venture a reconsideration of the significance of the pH measurement.

The conventional interpretation of the pH measurement was developed during the wide acceptance of the theory of incomplete ionization, and the resourcefulness of its exponents in the field of explanation would have been wholly commendable if the results obtained had led to a position from which other solution characteristics could have been predicted. The interpretation was based, obviously enough, upon the theory of incomplete ionization, and is so involved with that theory as to be untenable in its present form if associated with the theory of complete ionization.

During more recent years, however, as has been pointed out, an increasing body of evidence in support of complete ionization has been forthcoming. This evidence cannot be ignored, nor can we remain oblivious to the fact that this evidence is contrary to the conventional H^+ ion interpretation of pH measurements. Suppose, moreover, that important additional evidence in support of the theory of complete ionization was forthcoming, how would we then interpret the pH measurement?

Important additional evidence in support of the theory of complete ionization at all concentrations has been developed recently by the writer (**11, 12, 13**) from an extension of GRAHAM'S law to solute ions through the assumption of a periodic inverse integral hydration. There may be many not yet ready to acknowledge the admissability of this evidence because it involves the seemingly radical assumption of weight change with ioniza-

tion. It nevertheless becomes apparent that the specific hydration of solute ions not only permits the demonstration of complete ionization with the very data of electrical conductivity and freezing-point depression which were so largely responsible for the development of the theory of incomplete ionization, but, what is more important, permits the prediction of many solution characteristics at all concentrations. Heretofore we have never had a solution theory which proved at all satisfactory in concentrated solutions. Naturally there can be no question but that the new view-point will have its own limitations in its application to solution phenomena, but the extent to which it has already lent itself to prediction appears to warrant the study of its possible significance in relation to various phases of solution phenomena. At this time one of the most studied of these phases is acid-alkali reactivity. If the conventional interpretation of pH is the correct one, surely the relationship between solute and solvent will emphasize that correctness. If the conventional interpretation of pH now seems erroneous, however, let us cheerfully acknowledge our past mistakes and set about making our readjustments to a modified view-point with that zest which has always made research high adventure. Moreover, if our studies revive the ghost of PROUT and hold out a wholesome simplicity to chemical science, let us not flinch before the academic prestige of the printed page and inherited interpretations. The chemist with the modern concept that ideas must be complex in order to be valid will not be interested in this paper. Yet as physiologists may we not be permitted to acknowledge our disappointment in the conventional interpretation of the pH measurement and to question its correctness? When we lose the power and courage to face the facts and think independently we will have become useless for research, and may as well turn our work over to regimented robots.

The new description of hydration

There may be those who have some misgivings in regard to the existence of an attractive force between solute and solvent which is termed hydration, but in general such a force has long been widely recognized. JONES and his associates (15) took an important part in bringing about this recognition through the establishment of several independent lines of evidence all pointing to the existence of hydrates in solution. Whether or not one accepts their further description of the relationship (and it now appears demonstrable as invalid), one may scarcely venture to deny the existence of the attractive force, particularly since various other researches during the last half-century have similarly indicated its existence.[1] Without desiring to seem arbitrary in this matter, it seems best not to complicate the

[1] A valuable series of papers on hydrates in solution will be found in Trans. Faraday Soc. 3, part 2. Oct. 1907.

discussion at this point with a comprehensive examination of the evidence for hydration, but rather to accept the findings of these various workers to the extent of subscribing to the existence of an attractive force between solute and solvent, restricted, of course, to those solutes and solvents in which the force has been so thoroughly demonstrated. A detailed study of the researches on hydration leads almost inevitably to the conclusion that such a view-point is justified.

With particular reference to acidity and alkalinity, however, it is to be noted that the researches on hydration demonstrate the existence of little or no attractive force between an H^+ or an OH^- ion and an aqueous solvent (**15, 22**). These ions are not the only exceptions, for the somewhat analogous SH^- and S^{--} ions appear similarly characterized; but it is of major concern to us that the H^+ and OH^- ions seem to be outstanding representatives of a small group in which practically no attraction of the sort designated as hydration can be demonstrated between the ions and their aqueous solvent.

Against the background of solute ions for the most part characterized by an attraction for their aqueous solvent, yet with a few characterized by the absence of such an attraction, we may now take up the more detailed description of the attraction called hydration as applied to dissolved substances. There have been a number of attempts to extend the description of hydration beyond the abstract status of an unevaluated force, and it may be well worth while to review these studies at this point. Yet to bring something of conciseness to such a review it seems best to select an electrolyte as representative and standard. The electrolyte KCl in aqueous solution is the conventional standard for studies of the electrical conductivity of solutions, and appears suitable to serve likewise as a standard for studies of hydration. We may now turn to the experimental data, therefore, and examine the attraction there indicated as existing between the solute KCl and its aqueous solvent.

Using the depression of the solubility of a gas accompanying the addition of an electrolyte as an index of the amount of bound water, or water associated with the electrolyte through hydration, the results summarized in table I were obtained by KNOPP (**16**), STEINER (**23**), PHILIP (**20**), and GORDON (cited by ARMSTRONG and CALDWELL **1**).

The results given in table I indicate that, on the basis of the method there represented, about ten molecules of water are associated with each molecule of KCl in aqueous solution. The results obtained by other methods are in less satisfactory agreement among themselves, but nevertheless of interest. BOUSFIELD (**4, 5**), working with observed relative migration velocities of ions as indices of hydration, obtained 12 as the specific molecular hydration of KCl in his earlier work, and 9 in his later work,—values of the order in-

TABLE I

SPECIFIC MOLECULAR HYDRATION OF KCl AS INDICATED BY THE EFFECT OF THIS SOLUTE UPON REDUCTION OF SOLUBILITY OF GASES IN SOLVENT

PERCENTAGE KCl BY WEIGHT	INVESTIGATOR	° C.	AVERAGE SPECIFIC MOLECULAR HYDRATION (NUMBER OF WATER MOLECULES ASSOCIATED WITH EACH MOLECULE OF SOLUTE)
1.09	KNOPP	20	9.8
2.12	KNOPP	20	11.1
3.83	STEINER	15	10.5
4.07	KNOPP	20	10.0
6.37	KNOPP	20	10.0
7.38	KNOPP	20	10.0
7.45	PHILIP	15	9.4
7.45	GORDON	15	9.9
7.48	STEINER	15	9.4
		Average	10.011

dicated in table I. CALDWELL (**8**), working with the accelerating influence of salts on actions in which acids were concerned, obtained 10 as the specific molecular hydration of KCl, a value in excellent agreement with the values in table I. In the work of six investigators using three methods there is thus to be observed a substantial concurrence of evidence that in an aqueous solution of KCl ten molecules of water are associated with each molecule of solute. Other results appear widely scattering. JONES and his associates (**15**), from a study of so-called "abnormal" freezing-point depressions, concluded that there was no hydration in solutions of KCl; while REMY (**21**), from considerations of supposed ionic radii in conjunction with observed ion mobilities, obtained 32 as the specific molecular hydration of KCl in aqueous solution. Obviously the methods used by these investigators must be held open to question, and until contrary additional evidence is brought forward we may scarcely do better than to conclude that 10 molecules of water are associated with each molecule of KCl in aqueous solution. It is important to note that this value is a whole number, and it will be recalled that the number of molecules of water observed as characterizing hydrated salts crystallizing out of aqueous solutions is usually likewise subject to expression in whole numbers per salt molecule.

The observational data relating to the specific molecular hydration of KCl will scarcely permit further conclusions at this point, but some additional findings are suggestive and of interest. ABEGG and BODLÄNDER (**2**) pointed out that, restricted to salts giving rise to a common negative ion,

hydration is inversely related to the atomic weight of the positive ion. This relationship was further corroborated and emphasized in the work of SENTER (**22**), BOUSFIELD (**5**), and JONES (**15**). BOUSFIELD further pointed out that the specific molecular hydration of the Cl^- ion is greater than that of the K^+ ion, and that the specific molecular hydration of the Na^+ ion is about four times that of the K^+ ion. There can be no doubt but that these investigators recognized the importance of the foregoing relationships, in particular the inverse relation between hydration and atomic weight, italicized in the work of JONES. The relationships, nevertheless, remained sterile until they became incorporated in the extension of GRAHAM'S law to solute ions and contributed to what may be called the new description of hydration.

Under the gas diffusion law of GRAHAM the velocity of a molecule is described as inversely proportional to the square root of its density:

$$V_1 : V_2 :: \sqrt{D_2} : \sqrt{D_1}$$

Combining this with the AVOGADRO gas law:

$$MW_1 : MW_2 :: D_1 : D_2$$

we have

$$V_1 : V_2 :: \sqrt{MW_2} : \sqrt{MW_1}$$

or the velocity inversely proportional to the square root of the molecular weight, a familiar and widely recognized relationship. Extending this relationship to solute ions we have

$$V_1 : V_2 :: \sqrt{IW_2} : \sqrt{IW_1}$$

or the velocity inversely proportional to the square root of the ionic weight. JONES (**15**) stated it as an established fact that the velocities of the ions were an inverse function of their mass, while BREDIG (**6**) found that the conductance of an element ion was a periodic function of its atomic weight. Applying the inverse relationship to the observed ionic mobilities of the K^+ and Na^+ ions (for example, at 18° C. $K^+ = 64.5$, $Na^+ = 43.3$ (10)) through the tentative addition of whole numbers of water molecules to these ions, and incorporating the observed specific molecular hydration observed for KCl, and also the suggestive additional findings above noted, we eventually derive an inverse *integral* relationship between ionic weight and hydration. Since the integral feature becomes incorporated, it follows inevitably that the ionic weights must have a corresponding regularity not afforded by the observed atomic weights, but rather such as that obtained by doubling the atomic numbers. These developments, though perhaps somewhat abstruse

as here abbreviated, and certainly unconventional however presented, really become illuminating when the observed ionic mobility of the H^+ ion, considered as unhydrated, is brought into the system; for it then becomes obvious that the basic weight assigned to the element through doubling its atomic number has been modified incident to, and in a direction depending upon, the ionization. The observed ionic mobility of the K^+ ion thus becomes evidence, under GRAHAM'S law and the foregoing observed relationships, that the K^+ ion of weight 2×19 (A. N.) $+2$ (positive charge), or 40, hydrates with 3 water molecules and moves as though of weight 94 ($40 + 3 \times 18 = 94$), and that the Cl^- ion of weight 2×17 (A. N.) -2 (negative charge), or 32, hydrates with 7 water molecules and moves as though of weight 158 ($32 + 7 \times 18 = 158$).

Unless one is in a position carefully to work out for himself the seemingly involved mathematical considerations introduced in the foregoing extension of GRAHAM'S law, it may be difficult to attain assurance of the integrity of the above interpretation, since there are a number of new ideas involved, some of which appear disturbing to the view-point to which we have long been accustomed. Moreover, in such a procedure many investigators would soon feel the need of a more detailed description than can possibly be included herewith in a paper on acidity and alkalinity. But after all the important thing about a description is not how one derived it, but what can it do; so we may proceed to outline some of the end results of the new description in so far as they immediately involve our base value ions, K^+ and Cl^-, and then venture prediction, the ultimate and only satisfactory test of any description being its serviceability in such a capacity. The new relationships embodying the weight range of the lighter elements are brought together in table II.

It may be observed that table II embodies most of what is known about hydration in relation to the involved elements. The associated water is expressed in whole numbers, analogous to the situation in hydrated salts crystallizing out of aqueous solutions. The associated water is also inversely related to atomic weight, noted and reported by several investigators as an outstandingly important relationship. The water associated with KCl totals 10 molecules, the value noted in the data of table I. The Cl^- ion with 7 water molecules is more hydrated than the K^+ ion with 3, as observed by BOUSFIELD, and the hydration of the Na^+ ion, 11 water molecules, is about four times that of the K^+ ion, 3 water molecules, as also observed by BOUSFIELD.

In proceeding to test the validity of the relationships represented in table II, it should be borne in mind that the values have been derived through studies of solute ions and thus pertain only to solute ions on the basis of observed measurements considered up to this point. It will readily

TABLE II

WEIGHT, HYDRATION, AND VELOCITY VALUES CALCULATED FOR THE LIGHTER ELEMENTS THROUGH AN EXTENSION OF GAS LAWS IN RELATION TO OBSERVED ELECTRICAL CONDUCTIVITIES

A.N.	E.	Assumed as weight 2 × A.N.	Velocity if not hydrated V_1	Postulated number of water molecules	Molecular weight of hydrated ion	Velocity if hydrated V_2	Ionic weights and velocities of the common hydrated ions			
							Ion	Weight		Velocity
								Anhydrous	Hydrated	Hydrated
0		0		23	414	491				
1	H	2	7082	22	398	501				
2	He	4	5000	21	382	512				
3	Li	6	4090	20	366	523				
4	Be	8	3546	19	350	535	Li^+	8	350	535
5	B	10	3162	18	334	547				
6	C	12	2887	17	318	561				
7	N	14	2672	16	302	575				
8	O	16	2500	15	286	591	F^-	16	286	591
9	F	18	2357	14	270	609				
10	Ne	20	2236	13	254	627				
11	Na	22	2132	12	238	648				
12	Mg	24	2041	11	222	671	Na^+	24	222	671
13	Al	26	1961	10	206	698				
14	Si	28	1890	9	190	726	Mg^{++}	28	190	726
15	P	30	1826	8	174	758				
16	S	32	1768	7	158	796	Cl^-, Al^{+++}	32	158	796
17	Cl	34	1715	6	142	839				
18	A	36	1667	5	126	891				
19	K	38	1622	4	110	953				
20	Ca	40	1581	3	94	1031	K^+	40	94	1031
21	Sc	42	1543	2	78	1133				
22	Ti	44	1508	1	62	1271	Ca^{++}	44	62	1271
23	V	46	1474	0	46	1474				

become apparent that, in the event these relationships are satisfactorily substantiated, the conventional observed weight data become subject to reexamination for an alternative interpretation; but this aspect is entitled to individual consideration and will be reserved for such treatment. For the present, therefore, we shall use the new weight values without apology, but leave the reader free to substitute the conventional observed weight values at his own risk.

We may establish our basic values for the electrolyte KCl in aqueous solution most readily from table II by selecting 1000 grams as the unit of solution and one gram molecule as the unit of solute. On this basis we derive

$K^\circ = 38$,	$K^+ = 39$,	$K^{[+]} = 40$	hydrates with 3	$Cl^{[-]h} = 158$	$K^{[+]hv} = 1031$
$Cl^\circ = 34$,	$Cl^- = 33$,	$Cl^{[-]} = 32$	hydrates with 7	$K^{[+]h} = 94$	$Cl^{[-]hv} = 796$
KCl 72	72	72	10	252	1827

The exponential zero above signifies the neutral atom. In so far as we are aware such an atom does not exist in solution; probably it does not exist in the solid state, certainly not in the KCl crystal; and possibly it does not exist at all as a stable condition. Nevertheless the concept is inevitable under weight change with ionization. In the case of the Cl atom above, for example, the ionic form Cl^{+++++} preceding the formation of the radical ClO^-_3 must be considered as having had a combining weight of 39; and at one point in the transition from the atomic state Cl^{+++++} to Cl^- there must have been neutrality. In a substantial measure the irregular observed weight values appear to have resulted from the failure to recognize weight change with ionization, and the consequent attempt to make one value represent the activity of various ions; but that is another story.

When we are forced to conclude that weight change accompanies ionization, therefore, we obviously mean that we must consider such a change simply as an adjunct of being dissolved, not, as might be thought, the change attendant upon the addition of a discrete particle. In the compound KCl, for example, the K^+ and Cl^- ions are to be conceived as sharing a single electron (or a pair, if one prefers, depending upon the assigned weight values for the change) by virtue of which sharing the *combining* weight of the K^+ ion, represented in the preceding discussion simply by an exponential plus sign, becomes subject to evaluation as 39, while the combining weight of the Cl ion becomes subject to evaluation as 33. Upon release through solution, however, we have an increase in the effective positive charge in the K^+ and a corresponding increase in the effective negative charge of the Cl^-. Weight and positive charge thus become synonymous, a relationship which conventional chemistry denies to all elements except possibly the elements carbon, oxygen, and helium.

Weight now becomes resolvable into three categories: (1) neutral weight, not subject to measurement as yet; (2) combining weight, subject to measurement through combining ratios; and (3) ionic weight, subject to measurement through velocity under GRAHAM'S law. It is this ionic weight as indicated by velocity that has given us the relationships of table II, and, conversely, it is velocity predicted by expected ionic weight that is to become of interest in relation to acidity and alkalinity. The ionic state in this paper is designated by a bracketed exponent, followed in the case of hydration by a small *h* but otherwise signifying no hydration. The velocity is designated by an added small *v*.

From the preceding data relating to a specified solution of KCl we may venture to calculate a number of its expected characteristics. The weight of the hydrated solute being 252 grams with the total weight 1000 grams, it follows that the difference, 748 grams, is solvent. At this concentration, therefore, the weight of solvent is 74.8 per cent. of its weight at a zero concentration of solute. If this hitherto unevaluated change in concentration associated with hydration is correct, then the apparent decrease in specific molecular electrical conductivity and freezing-point depression, associated with an increase in concentration and interpreted as evidencing incomplete ionization, becomes attributable to such a change in concentration if the decrease is predictable on the new basis. If the observed values become constant following the correction for such a change, moreover, it follows that complete ionization must characterize the solution. In view of the increasing amount of evidence indicating such a complete ionization, we may venture to interpret the apparent rate of decrease in specific molecular electrical conductivity and freezing-point depression as an index of hydration. Conversely we may venture to predict, on such a basis, the apparent rate of decrease. Reference to observed data for aqueous solutions of KCl will reveal that the observed decreases in specific molecular electrical conductivity and freezing-point depression for solutions corresponding in concentration to 1.0 molecular, as previously described, are of the order of 74.8 per cent. (**3, 19, 11**). The conclusion is inevitable that hydration brings about an increase in concentration, and that the failure to recognize and correct for this increase in concentration contributed to the formulation of the theory of incomplete ionization.

From the foregoing data on KCl we may also venture to predict other solution characteristics, including the specific gravity, the capacity for gas absorption, and the transfer numbers of the ions. Yet in view of the results cited previous to the extension of GRAHAM'S law and the results just noted, there seems to be no real point in the further demonstration of the hydration characteristic of the solute ions K^+ and Cl^-. This is particularly true in that the velocity values for the hydrated K^+ and Cl^- ions, and the sum-

mation of these values, will be subsequently employed in considering some of the more dynamic aspects of solution phenomena.

Having already devoted considerable space to the manner in which the values of table II may be used in demonstrating and predicting static phases of solution phenomena, the reader must be left to satisfy himself with respect to the corresponding treatment of aqueous solutions of NaCl, LiCl, KF, NaF, $CaCl_2$, $MgCl_2$, and $AlCl_3$, all of which will be found substantiating the inverse integral hydration system (**3, 19, 11**). We may next turn our attention to the dynamic phases of solution phenomena, using an aqueous solution of KCl as a basis for the consideration of the velocity values derived from the extension of GRAHAM'S law in connection with the inverse integral hydration thus substantiated.

Velocity of solute ions in relation to acidity, alkalinity, and pH

In the foregoing discussion we have dealt with hydration in its effect upon the relative concentration of solute and solvent, and we may now turn our attention more particularly to the solute itself. We derived a summation velocity value of 1827 for the hydrated K^+ and Cl^- ions, and in a similar way table II supplies us with corresponding values for other electrolytes. For example:

$Li^o = 6$, $Li^{[+]} = 8$ hydrates with 19 water molecules $Li^{[+]h} = 222$, $Li^{[+]hv} = 535$
$Na^o = 22$, $Na^{[+]} = 24$ hydrates with 11 water molecules $Na^{[+]h} = 350$, $Na^{[+]hv} = 671$
$F^o = 18$, $F^{[-]} = 16$ hydrates with 15 water molecules $F^{[-]h} = 286$, $F^{[-]hv} = 591$

$Li^{[+]hv} = 535$	$Na^{[+]hv} = 671$	$K^{[+]hv} = 1031$
$Cl^{[-]hv} = 796$	$F^{[-]hv} = 591$	$F^{[-]hv} = 591$
Total = 1331	Total = 1262	Total = 1622

With these totals or summation values we may now venture to predict the specific molecular electrical conductivities of any of the given electrolytes from an observed value, temperature and concentration being understood as constant, through solving the equation

$$1827 : 1262 : : 145^2 : x$$

KCl : NaF : : KCl : NaF, etc.

$x = 100.158$. Observed value = 99.75.[2]

Using this method it will be found that the observed values for all salts giving rise to monovalent ions of the lighter elements give the predicted conductivities (**11**). If suitable allowances are made for differences in concentration and for the relationship between velocity and conductivity in polyvalent ions, electrolytes comprising the ions Mg^{++}, Ca^{++}, and Al^{+++} may be added to the list. It thus becomes evident that the inverse integral hydration is further substantiated by dynamic phases of solution phenomena beyond the basic values responsible for its development.

[2] Observed values at 0.001 mol. (new weights) and 25° C. by writer.

We may now consider the dynamic phases of the H^+ and OH^- ions, observed as exceptional in that they gave no evidence of being hydrated. From table II we may derive the following velocity values:

$$K^{[+]hv} = 1031$$
$$H^{[+]v} = 5000$$
$$OH^{[-]v} = 2357 \text{ (for 18)}$$
$$= 2500 \text{ (for 16)}$$

We may then venture to predict relative ion conductance at 18° C. as follows, 64.5 being the observed value for the K^+ ion (**10**):

(1) $K^{[+]hv} : H^{[+]v} : : K^{[+]hv} : H^{[+]v}$

$1031 : 5000 : : 64.5 : x \qquad x = 313$

The observed ion conductance of the H ion at 18° C. is 313 (**10**), and the agreement substantiates the other evidence that the H^+ ion is characteristically unhydrated.

(2) $K^{[+]hv} : OH^{[-]v} : : K^{[+]hv} : OH^{[-]v}$

For 18, $1031 : 2357 : : 64.5 : x \qquad x = 148.5$

For 16, $1031 : 2500 : : 64.5 : x \qquad x = 156.5$

The observed ion conductance of the OH^- ion at 18° C. is 174 (**10**). The agreement is not satisfactory, and yet is sufficient clearly to indicate that the OH^- ion is not hydrated, for in the latter case the ion conductance would be of the order of 37. Granting the fact of an exception in this instance, we may tentatively consider 16 to be the expected anhydrous weight of the OH^- ion and proceed with the value 2500 as representing its velocity referred to the base KCl = 1827.

It now becomes of interest to compare the relative velocities of individual ion components of various electrolytes in accordance with the foregoing considerations, and in such a comparison to search for a means of expressing relative velocities in such a way as possibly to serve as an index of the solution characteristic designated as acidity or alkalinity. If we obtain the ratio of the velocity of the faster moving ion to that of the slower ion by simple division, and prefix this by the sign of the faster ion, we have a system of evaluation in which equal velocities are represented as 1.0. Subtracting 1.0 from this value we then have a system in which equal velocities are represented by zero. Such a base would then correspond to neutrality in case acidity and alkalinity were associated with differential activities of the respective positive and negative ions. As examples we have

$H^{[+]v} = 5000$ $Cl^{[-]hv} = 796$ $5000 \div 796 = 6.28$ $6.28 - 1 = 5.28$; ion of greater velocity is positive. Acid-alkali index for HCl = + 5.28 (acid). $Li^{[+]hv} = 535$ $OH^{[-]v} = 2500$ $2500 \div 535 = 4.67$ $4.67 - 1 = 3.67$; ion of greater velocity is negative. Acid-alkali index for LiOH = − 3.67 (alkaline).

In extending this study to include various other electrolytes, it soon becomes apparent that in the polyvalent ions the number of charges is a factor

in the acid-alkali activity. At this point we may grant another exception and tentatively multiply the velocity values by the numbers representing the valence of the ions. The velocity-charge products thus obtained may now be treated as were the simple velocity values to derive indices of acid-alkali activity. In order to comprehend a truly representative series of electrolytes over the acid-alkali range, however, it becomes necessary to extend the inverse integral hydration system, noted in table II as characterizing the weight range of the elements of the first quarter of the periodic system. This hydration system as thus extended and previously published (**12**) becomes periodic with four phases, thus differing somewhat from the periodicity noted by BREDIG (**6**); and it is important to note that under conditions in which ions hydrate, such hydration appears to be a simple function of weight, independent of the nature of the ion. It thus develops that hydrating radicals of a weight comprised within the weight range of the elements may be treated as element ions of the same weight. In so far, therefore, as we are able to classify ions into the respective groups hydrated and unhydrated, we may now venture to predict, from the index obtained through the use of velocity-charge products, the relative acid-alkali reactivity of a solute electrolyte. In table III a number of electrolytes representing the acid-alkali range have been brought together to permit a comparison of their relative positions on the basis of the velocity-charge product index and on the conventional basis designated as pH.

In table III it will be observed that the order of acidity-alkalinity of the electrolytes as derived from a consideration of relative velocity-charge products corresponds with that derived from conventional measurements, except as regards sodium hydroxide, where a partial breakdown of the OH^- ion is suggested. In the conventional measurements, however, the degree of acidity-alkalinity is commonly interpreted as representing the actual presence of a certain concentration of H^+ and OH^- ions. The differences in solutions with respect to their behavior toward indicators or electrodes may well be correlated with certain concentrations of H^+ and OH^- ions *when these ions and these alone are present.* A comparison of the values in columns 7 and 8 of table III, however, leads to the suggestion that in aqueous solutions the velocity-charge products of the solute ions may be responsible for the activity conveniently attributed to specific H^+ and OH^- ion concentrations. The foregoing suggestion does not appear to be at variance with the recognized dominance of the H^+ ion in acidity. For example, we may note the velocity-charge products in some representative hydrogen-replacement series as brought together in table IV.

In table IV it will be noted that replacement of hydrogen uniformly reduces acidity as represented by the velocity-charge product and that alkalinity may result from such replacement.

TABLE III

COMPARISON OF TENTATIVE ACIDITY-ALKALINITY INDEX DERIVED FROM VELOCITY-CHARGE PRODUCTS WITH RELATIVE VALUES OF pH AS CONVENTIONALLY MEASURED

ELECTROLYTE	ASSUMED IONIZATION AND HYDRATION	CALCULATED IONIC WEIGHTS	CALCULATED IONIC VELOCITIES	CALCULATED VELOCITY-CHARGE PRODUCTS		TENTATIVE ACIDITY-ALKALINITY INDEX	OBSERVED* pH IN N/10 SOLUTION
				+ Ion	+ Ion		
HCl	$H^{[+]}$	$H^{[+]} = 4$	$H^{[+]} = 5000$	5000	796	+5.280	1.0
	$Cl^{[-]h}$	$Cl^{[-]h} = 158$	$Cl^{[-]h} = 796$				
H_2CO_3	$H^{[+]}\ H^{[+]}$	$H^{[+]} = 4$	$H^{[+]} = 5000$	5000	1026	+3.87	3.8
	$CO_3^{[--]h}$	$CO_3^{[--]h} = 380$	$CO_3^{[--]h} = 513$				
H_3BO_3	$H^{[+]}\ H^{[+]}\ H^{[+]}$	$H^{[+]} = 4$	$H^{[+]} = 5000$	5000	1482	+2.375	5.2
	$BO_3^{[-3]h}$	$BO_3^{[-3]h} = 412$	$BO_3^{[-3]h} = 494$				
$NaHCO_3$	$Na^{[+]h}$	$Na^{[+]h} = 222$	$Na^{[+]h} = 671$	671	1290	−0.923	8.4
	$HCO_3^{[-]}$	$HCO_3^{[-]} = 60$	$HCO_3^{[-]} = 1290$				
Na_2CO_3	$Na^{[+]h}$	$Na^{[+]h} = 222$	$Na^{[+]h} = 671$	671	2672	−2.98	11.6
	$CO_3^{[--]}$	$CO_3^{[--]} = 56$	$CO_3^{[--]} = 1336$				
NaOH	$Na^{[+]h}$	$Na^{[+]h} = 222$	$Na^{[+]h} = 671$	671	2500	−2.72	13.1
	$OH^{[-]}$	$OH^{[-]} = 16$	$OH^{[-]} = 2500$				
NaOH	$Na^{[+]h}$	$Na^{[+]h} = 222$	$Na^{[+]h} = 671$	591.5 (average)	5774	−8.76	13.1
	$Na^{[+]h}$	$H^{[+]} = 382$	$H^{[+]h} = 512$				
	$O^{[--]}$	$O^{[--]} = 12$	$O^{[--]} = 2887$				

* The ABC of hydrogen ion control. LaMotte Chemical Products Co., Baltimore, Md. 1926. p. 15.

TABLE IV

COMPARISON OF CALCULATED AND OBSERVED VALUES FOR ACIDITY AND ALKALINITY IN AQUEOUS SOLUTIONS OF CERTAIN ELECTROLYTES

Electrolyte	Assumed ionization and hydration	Calculated ionic weights	Calculated ionic velocities	Calculated velocity-charge products		Tentative acidity-alkalinity index	Observed (7) relative acidity-alkalinity reaction
				+ Ion	− Ion		
H_3PO_4	$H^{[+]}$	4	5000	5000	460	+ 9.87	Strong acid
	$H_2PO_4^{[-]h}$	474	460				
$H_2PO_4^-$	$H^{[+]}$	4	5000	5000	890	+ 4.62	Acid
	$HPO_4^{[--]h}$	506	445				
HPO_4^{--}	$H^{[+]}$	4	5000	5000	2697	+ 0.855	Weak acid
	$PO_4^{[-3]h}$	124	899				
NaH_2PO_4	$Na^{[+]h}$	222	671	671	460	+ 0.458	Extremely weak acid
	$H_2PO_4^{[-]h}$	474	460				
Na_2HPO_4	$2\ Na^{[+]h}$	222	671	671	890	− 0 326	Weak alkali
	$HPO_4^{[--]h}$	506	445				
Na_3PO_4	$3\ Na^{[+]h}$	222	671	671	2697	− 3.02	Alkali
	$Po_4^{[-3]h}$	124	899				

On the other hand an analogous study of alkalinity does not lead to the suggestion that the hydroxyl ion, OH^-, has a correspondingly dominant rôle in alkalinity; but rather that the oxygen ion, O^{--}, is the most active of the common negative ions. This suggestion was evidenced by the NaOH relationships in table III. Through analogy between sulphur and oxygen, further comparisons may be made as in table V.

An examination of table V with reference to alkalinity shows the following velocity-charge products for H_2O in the ionization assumed:

$$H_2O \text{ as } H^+,\ OH^- = +1.00$$
$$OH^- \text{ as } H^+,\ O^{--} = -0.155$$

These values suggest that with the assumed breakdown of the OH^- ion an amphoteric activity of the range $+1.00$ to -0.155 may be ascribed to unhydrated H^+, OH^-, and O^{--} ions in water, on which basis it would appear that the reaction of water should theoretically be slightly acidic rather than neutral.

Following up this suggestion it is of interest to go back to our observed ion conductance value for the OH^- ion at 18° C., 174. If there is the breakdown of the OH^- ion assumed as accountable for the amphoteric activity of water, the velocity-charge products represented would be $OH^- = 2500$ and $O^{--} = 5774$. Per unit electrical charge, the average value would thus be 2758. If we now use this value instead of the value $OH^- = 2500$ to predict the ion conductance of the so-called OH, we have

$$K^{[+]hv} : OH^{[-]v} : : K^{[+]hv} : OH^{[-]v}$$
$$1031 : 2758 : : 64.5 : x$$

Solving, we obtain 172.5, a value in substantial agreement with the observed value 174. It is fully realized that these and the foregoing considerations with respect to the reactivity of the ions of water are in the nature of tentative assumptions; and yet it is obvious that they have an intimate association with observed facts through the medium of amphoteric activity, ion conductance, and normally slight preponderance of "acidity." The conventional interpretation of pH does not have any obvious association with these facts and practically overrides experimental data in assigning a normal neutrality to water. It further becomes obvious that many electrolytes giving rise to none but hydrated ions in aqueous solutions would have an acid-alkali activity falling within the foregoing amphoteric activity of water, and would thus *appear* to be "neutral" salts. The "neutrality" of these salts has long been recognized as only relative, however, while their differing actions as to the modification of pH readings is also familiar. The rôle of buffers in the new description of hydration as related to acid-alkali reactivity is most intriguing, but cannot be taken up at this time.

Generalizing from the foregoing comparisons, it appears that when aqueous solutions of electrolytes are characterized by hydrated ions their

TABLE V

COMPARISON OF CALCULATED AND OBSERVED ACIDITY-ALKALINITY VALUES FOR CERTAIN ELECTROLYTES

Series	Electrolyte	Assumed ionization and hydration	Calculated ionic weights	Calculated ionic velocities	Calculated velocity-charge products		Tentative acidity-alkalinity index	Observed (10) reaction
					+ Ion	− Ion		
Sulphur	H_2S	$H^{[+]}$	4	5000	5000	1826	+1.74	Weakly acidic
		$HS^{[-]}$	30	1826				
	HS-	$H^{[+]}$	4	5000	5000	3780	+0.32	Very weakly acidic
		$S^{[--]}$	28	1890				
	NaHS	$Na^{[+]h}$	222	671	671	1826	−1.72	Weakly alkaline
		$HS^{[-]}$	30	1826				
	NaS-	$Na^{[+]h}$	222	671	671	3780	−4.64	Alkaline
		$S^{[--]}$	28	1890				
Oxygen	H_2O	$H^{[+]}$	4	5000	5000	2500	+1.00	Assumed as neutral, but generally observed as slightly acidic
		$OH^{[-]}$	16	2500				
	OH-	$H^{[+]}$	4	5000	5000	5774	−0.155	
		$O^{[--]}$	12	2887				
	NaOH	$Na^{[+]h}$	222	671	671	2500	−2.72	Alkaline
		$OH^{[-]}$	16	2500				
	NaO-	$Na^{[+]h}$	222	671	671	5774	−7.60	Strongly alkaline
		$O^{[--]}$	12	2887				

reaction tends to be relatively neutral; that when the positive ions are not hydrated their reaction tends to be acidic; and that when the negative ions are not hydrated their reaction tends to be alkaline. As yet we have no information concerning the factors which condition hydration; yet the validity of an assumed state with respect to hydration may be checked by measurements of diverse phases of solution phenomena, as has been shown. When these measurements yield a consistent interpretation it would appear that the velocity-charge product may render a real service in the evaluation of the acid-alkali characteristic of solutions of electrolytes.

For some years now we have been measuring pH in all sorts of situations, in tissue fluids, in streams, in soils, etc., and have accumulated considerable literature as a result. Yet who among us feels that we are getting anywhere in this research? In one locality a certain legume does not thrive in a soil of a certain pH and we conclude that the soil is too acid; yet in another locality the plant may be thriving in a soil with a higher acidity. The literature includes many such examples of apparent inconsistency accompanying the interpretation of pH measurements involving unknowns. In other words, physiological research has amply demonstrated that, with relation to organisms at least, the pH requirement is not a constant thing.

Nor is physico-chemical research without evidence to this same end. Notwithstanding the alleged specificity of the hydrogen electrode, there appear to be substantial reasons for associating acid-alkali reactivity with *all ions present,* and if with all ions present, then variable with the nature of the ions. There is no question but that most solution characteristics (electrical conductivity, freezing point, boiling point, etc.) involve the participation of all ions present; moreover, that these characteristics fail to demonstrate the presence of any such H^+ and OH^- ion concentration as that essential under the conventional interpretation of pH. For example, an aqueous solution of HCl gives an electrical conductivity both representable and predictable as the sum of the ion conductances characterizing the H^+ and Cl^- ions respectively, the individual values being the same in other electrolytes, such as H_2SO_4 and KCl. The observed ion conductance of the OH^- ion is of the order of three times that of the Cl^- ion. Under the conventional interpretation, therefore, the assignment of a pH value to an aqueous solution of HCl requires the assumed presence of OH^- ions under conditions in which they are not demonstrable, and in which they would be expected to become demonstrable if present. We are familiar with the labored conventional explanation of what happens, but it is difficult to overlook the fundamental principle of ionization, an equal number of positive and negative charges. The régime of H^+ ions see-sawing with OH^- ions while all other ions disappeared by request for the time being has been a hey-day of hope, but little real progress in research has been made.

Are we not justified, therefore, in examining this other view-point? It has obvious limitations. It will not directly help one bit in the study of unknown substances, but it may at least enlighten us as to the futility of expecting to accomplish anything with acid-alkali measurements independent of a knowledge of the involved ions. In thus broadening our viewpoint to include all ions, however, we may put ourselves in a position to make worthwhile advances in research.

In conclusion the writer desires to point out with emphasis that *no criticism of the conventional methods of measuring acid-alkali reactivity is intended;* simply that the measurements thus derived may be subject to another interpretation.

Summary

A study of the ionic velocity-charge products of representative solute electrolytes suggests that acidity and alkalinity are solution characteristics accruing from the dominant activity of positive and negative ions respectively, and bear no essential relation to the presence of hydrogen or hydroxyl ions.

U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

LITERATURE CITED

1. ARMSTRONG, H. E., and CALDWELL, R. J. Studies of the processes operative in solutions. V. The discrimination of hydrates in solution. Proc. Roy. Soc. London. A **79**: 586–597. 1907.
2. ABEGG, R., and BODLÄNDER, G. Die Elektroaffinität, ein neues Prinzip der chemischen Systematik. Zeitschr. anorg. Chem. **20**: 453–499. 1899.
3. BATES, S. J. The calculation of equivalent conductance at infinit dilution. Jour. Amer. Chem. Soc. **35**: 519–535. 1913. (See p. 532).
4. BOUSFIELD, W. R. Ionengrössen in Beziehung zur Leitfähigkeit von Elektrolyten. Zeitschr. physikal. Chem. **53**: 257–313. 1905.
5. ————————. Ionic size in relation to molecular physics, together with a new law relating to the heats of formation of solid, liquid, and ionic molecules. Proc. Roy. Soc. London. A **88**: 147–169. 1912.
6. BREDIG, G. Beiträge zur Stöchiometrie der Ionenbeweglichkeit. Zeitschr. physikal. Chem. **13**: 191–288. 1894.
7. BRITTON, H. T. S. Hydrogen ions. Chapman and Hall, Ltd. London. 1932.
8. CALDWELL, R. J. Studies of the processes operative in solutions. I. The sucroclastic action of acids as influenced by salts and non-

electrolytes. Proc. Roy. Soc. London. A **78**: 272–295. 1906.

9. CLARK, W. M. The determination of hydrogen ions. Williams and Wilkins Co. Baltimore. 1920.
10. CREIGHTON, H. J. Principles and applications of electrochemistry. Vol. 1. John Wiley & Sons. New York. 1924.
11. FLINT, L. H. Hydration of the solute ions of the lighter elements. Jour. Wash. Acad. Sci. **22**: 97–119. 1932.
12. ————. The hydration of the solute ions of the heavier elements. Jour. Wash. Acad. Sci. **22**: 211–217. 1932.
13. ————. Unhydrated solute element ions. Jour. Wash. Acad. Sci. **22**: 233–237. 1932.
14. GORTNER, R. A. Outlines of biochemistry. John Wiley & Sons. New York. 1929.
15. JONES, H. C., *et al.* The freezing-point lowering, conductivity, and viscosity of solutions of certain electrolytes in water, methyl alcohol, ethyl alcohol, acetone, and glycerol, and in mixtures of these solvents with one another. Carnegie Inst. Washington. Pub. no. 180. 1913.
16. KNOPP, W. Über die Löslichkeitsbeeinflussung von Wasserstoff und Stickoxydul in wässerigen Lösungen verschieden dissoziirter Stoffe. Zeitschr. physikal. Chem. **48**: 97–108. 1904.
17. KNOX, J. A study of the sulphur anion and of complex sulphur anions. Trans. Faraday Soc. **4**: 29–49. 1908.
18. MICHAELIS, L. Hydrogen ion concentration. Williams and Wilkins Co. Baltimore. 1926.
19. NOYES, A. A., and FALK, K. G. The properties of salt solutions in relation to the ionic theory. II. Electrical transference numbers. Jour. Amer. Chem. Soc. **33**: 1436–1460. 1911. (See p. 1456.)
20. PHILIP, J. C. Influence of non-electrolytes and electrolytes on the solubility of gases in water. Trans. Faraday Soc. **3**: 140–145. 1907.
21. REMY, H. Beiträge zum Hydratproblem. I. Berechnung der Menge des die Ionen einhüllenden Wassers aus den elektrolytischen Beweglichkeiten. Zeitschr. physikal. Chem. **89**: 467–488. 1915.
22. SENTER, G. Discussion of methods suggested for determining degree of hydration. Trans. Faraday Soc. **3**: 146–152. 1907.
23. STEINER, P. Über die Absorption des Wasserstoffs im Wasser und in wässerigen Lösungen. Wied. Ann. **52**: 275–299. 1894.

INFLUENCE OF SHORTER LIGHT RAYS UPON ABSORPTION OF NITRATE BY THE YOUNG WHEAT PLANT[1]

W. E. Tottingham, H. L. Stephens, and E. J. Lease

(with two figures)

Introduction

In a previous publication (**11**) evidence was presented that radiations of highest frequency in the visible spectrum and lowest in the ultraviolet region promote absorption of nitrate by the young wheat plant. Those results indicated that yellow to violet rays also increase the synthesis of protein in this plant, while the longer ultraviolet do not. Popp (**9**) had already observed beneficial effects of the shorter visible rays of sunlight in the development of various plant species, but he concluded that these did not modify chemical composition of plant tissues appreciably and that the ultraviolet region is dispensable. Warburg (**12**) found indications of a complementary relation between respiration of carbohydrates and assimilation of nitrate in *Chlorella*. There seems to be lack of substantial demonstration that direct photochemical reduction of nitrate, known to occur *in vitro,* functions extensively *in vivo.*

The present work was planned to deal only with gross fractions of the spectrum, leaving more detailed investigation to those equipped for operation under selected wave lengths of light. Since the regulation of spectral portions of sunlight to equal intensity on a large scale without modifying the distribution of wave length is not readily accomplished, lamps have been used.

Experimentation

Wheat seed[2] was selected from a pure line stock of the Progress variety, this being an awned spring type resembling Marquis. After surface sterilization and soaking it was germinated between papers at about 20° C. When the plumules were emerging, seedlings of uniform appearance were fixed on paraffined wire netting suspended over the nutrient solution in battery jars. The latter were 14.3 cm. in diameter, with a capacity of 3.3 liters, and had been painted black on the exterior to exclude light. During the first day the plants were partially shaded to favor establishing of the root system, and three or four days thereafter the population was reduced to 50 per jar.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station. Supported by annual grants from the Committee on the Effects of Radiation upon Living Organisms, Division of Biology and Agriculture, National Research Council.

[2] Supplied through the kindness of Prof. B. D. Leith, of the Department of Agronomy, this institution.

The nutrient solution had a total concentration of 0.24 per cent., with distribution gram-molecularly as follows: 0.0010 $MgSO_4$, 0.0016 $Ca(H_2PO_4)_2$, 0.0040 NaCl, 0.010 KCl, 0.0090 $NaNO_3$, and about 0.00004 ferric citrate. Most of the salts were of a special lot prepared under specifications for the Committee on Salt Requirement of Plants, National Research Council (**6**). Others were C.P. brands but the NaCl and ferric citrate were of commercial grade. Distilled water was provided as condensate derived from the steam heating service of the greenhouse. This provided a solution which favored elongation and production of dry matter by the plants as much as did one prepared by redistilling the water in glass.

Following complete weekly renewal of the nutrient solution, the plants were transferred to chambers for climatic control 114 days after placing in the culture jars. A few series, as indicated by the data, had been raised under Mazda lamps but at the usual temperature range of 17°–20° C. For those grown in the greenhouse, average intensities of light for the effective length of day have been computed from records of the local station of the U. S. Weather Bureau. Use was here made of the proportion 1 hr. cal. = 140 f.c., as derived earlier (**10**).

The chambers employed for climatic control had been modified since their earlier description (TOTTINGHAM **10**), as shown in figure 1.[3] Use of the humidostat was discontinued, owing to occasional sticking and overheating of the damper-closing solenoids. In lieu of this service the damper between the blower and humidifying chamber was adjusted manually. A value of about 65 per cent. relative humidity of the atmosphere was adopted as favorable to the plants at the temperature effective (14.5°–16.5° C.). Difficulties during earlier tests had led to the adoption of artificial illumination exclusively. The intake of the humidifying fan was so connected as to draw outdoor air through the lamp dome above the culture chamber, this serving to supplement use of the water cells in reducing the heating effects of illumination. It also renewed the supply of CO_2 for the plants. While the degree of control over temperature and humidity was less satisfactory than that recorded earlier (**10**), it was possible to establish somewhat compensatory variations.

Four reflectored 500-watt Mazda C lamps suspended above the water cell in each chamber were operated for a period of 16 hours daily. Supplementary short wave radiation was provided in one chamber by use of a Thomsen arc lamp operating uninclosed at 7.5 amp. on 110 V. The rays were reflected horizontally by a vertical, parabolic mirror through common glass in the door of the chamber, thus screening out injurious shorter ultraviolet rays. The lamp carbons were 13 mm. in diameter, one being solid

[3] We are indebted to the General Electric Company for providing the double-contact magnetic switches used in connection with the heating units.

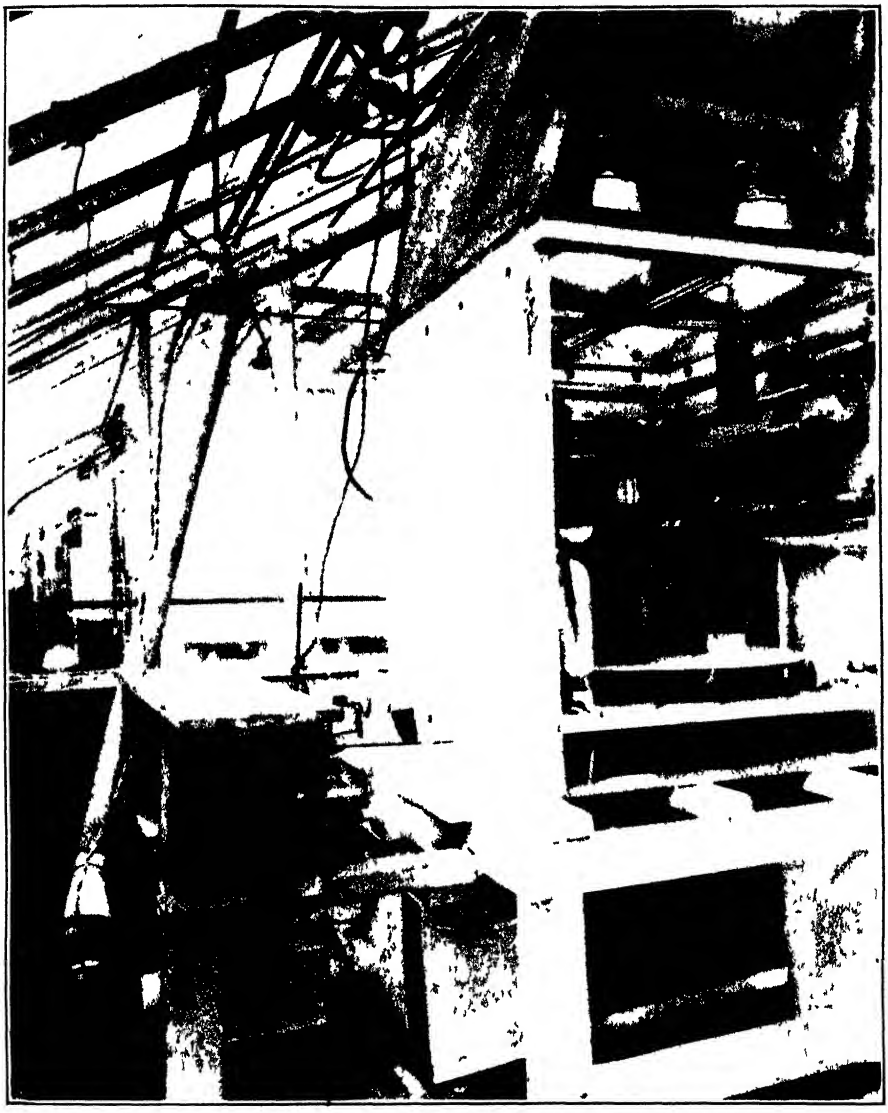


FIG. 1. Climatic chamber, with attachments: The hygrothermograph is riding on the elevated rotating table, as used for young plants. Hanging at the front is the thermostat, which operates luminous radiator units against the refrigerating coils. Note the ventilated lamp dome above the water cell and the humidifying chamber beneath the supporting table. The magnetic switch for heating current hangs on the back of the chamber and the voltage transformer for use with the thermostat is partly visible at the base of the chamber on the left.

and the other cored.[4] An equivalent increase in total radiation in the control chamber was provided by similar use of a 150-watt Mazda lamp. These adjustments, together with measurements of total intensity of light, were made by use of a thermopile receiver and suitable glavanometer.[5] Foot candle equivalents of the radiation were derived by comparison with a new 150-watt Mazda lamp and found to be 1600 f.c. in the starvation period, with 80 f.c. additional during use of the arc lamp, these values being taken as effective at the uppermost plane of horizontally disposed leaves.

For evaluating the general effect of supplementary use of the carbon arc upon the distribution of energy, comparative measurements[6] were made upon this lamp and the ordinary Mazda lamp. Screens of special glasses[7] were used for this purpose in conjunction with a water cell to absorb heat. One of these (Noviol A) transmitted essentially radiations greater than 4200 Å, thus eliminating ultraviolet and a large portion of the violet region of the spectrum. The other (G34 Y) transmitted essentially radiations longer than about 6000 Å, thus eliminating ultraviolet and shorter visible radiations up to the orange region. Results obtained in this manner are shown in table I, the values having been taken at the same distance (45 cm.) as was effective with the plants. These data show that the effective increase of ultraviolet and violet radiation by supplementary use of the carbon arc was only about 1.0 per cent. of the total radiation, or less than 4 per cent. of the spectral region in question. This is much less than our earlier estimate of increase (**11**, p. 2443), in which too high value was accredited an arc lamp of low amperage. There was a somewhat smaller increment in the total region of the radiations shorter than orange. Our screens fail to give a separate evaluation for blue and violet rays, which to the eye appear appreciably augmented by use of the arc. While recognizing that frequencies both favorable and unfavorable to absorption of nitrate may be included within any of the spectral regions here defined, we have proceeded upon the basis that a net effect of such a system should be determinable.

Tests with single salts

When transferred to the control chambers, the cultures were supplied with a nitrate-free solution formed by omitting this salt from the usual

[4] Appreciation is expressed for a gift of carbons from the National Carbon Company.

[5] The thermopile was supplied by the Eppley Company and the galvanometer by the Leeds Northrop Company.

[6] For advice and aid in these measurements we are indebted to Royce E. Johnson, director of the Electrical Standards Laboratory of this institution.

[7] Supplied by the Corning Glass Works. The transmissions of these glasses are given in Technologic Paper 148, U. S. Bureau Standards, 1920. Noviol A is covered by curve 23, fig. 7 and G34 Y by curve 11 × 26, fig. 23. Our plates were 4 mm. thick, however, and would therefore vary somewhat from the transmissions quoted.

TABLE I

INTENSITY AND DISTRIBUTION OF LIGHT EMITTED FROM THE MAZDA LAMP AND CARBON ARC

Light source	Spectral regions						Distribution of total light in regions of shorter radiations	
	3100 to 7600 Å		4200 to 7600 Å		6000 to 7600 Å			
	Galvanometer deflection	Proportion of total light	Galvanometer deflection	Proportion of total light	Galvanometer deflection	Proportion of total light	Shorter than blue	Shorter than orange
	cm.	%	*cm.*	%	*cm.*	%	%	%
110 V. a.c., 500-watt Mazda lamp	52.9	100	38.4	73	33.8	64	27	36
110 V. a.c., 7.5 amp.* carbon arc	18.9	100	11.0	58	10.1	53	42	47
4 Mazda lamps supplemented by carbon arc†	162.5	100	117.4	72	103.4	64	28	36

* Average of 5 maximum and 5 minimum readings.

† Computed on the basis of a 20 per cent. time relation of the arc to the Mazda lamps, and factor of three for average intensity of four Mazda lamps as placed.

solution. This medium was supplied for a period of four days, during which time the plants were allowed to become adjusted to the new climatic environment. A preliminary test had demonstrated that all nitrate disappeared from plants starved under similar conditions. For an additional final period of three days the plants were subjected to a supply of nitrate only. This practice appeared to be justified by the results of GERICKE (3), who found wheat well nourished by rotation of the usual nutrient salts one by one in intervals of a few days each. It was assumed that this procedure would minimize the influences of other ions upon absorption of NO_3. In order to avoid abrupt changes in osmotic relations between solution and plant, the single nitrates were supplied in concentrations approximating that of the complete nutrient solution. Thus, the solution of KNO_3 contained 0.225 per cent. salt, equivalent to 0.31 mg. N per cc., and the other nitrates were used in equi-normal concentrations.

At the end of the test period the roots were rinsed into the residual solution and the latter was restored to its original volume, for the determination of NO_3. Aliquots of the unplanted nutrient solution were similarly analyzed to provide a base line for the expression of absorption values. The nitrate determination was carried out by the colorimetric procedure of HARPER (5). To provide a basis for relating absorption rates to the organism, the weight of the entire plants in each culture was determined after drying at 98° C. In most cases the effect of a given treatment represents the average of two or more cultures.

In two tests comparison was made of the nitrate carriers under the combined lamps only. These gave unusually high absorption from KNO_3 (53 and 61 mg. N per culture), and in each case 55 per cent. as great absorption from $NaNO_3$. One of these trials included $Ca(NO_3)_2$, with an absorption only 16 per cent. of that from KNO_3. Calcium was included in seven other tests and magnesium in two. These carriers proved relatively inefficient, giving no absorption of nitrate in some cases. No consistent relation of these effects to either the plane of preparatory illumination or the temperature of the test was apparent. A record of those tests in which the responses with sodium and potassium nitrates were compared under the two types of radiation is presented in table II. Dates are included to serve as indices of the character of solar radiation during the preparatory growth period.

From these data (table II) it appears that the range of nitrate absorption per unit of tissue was rather wide, namely, 15 to 31 mg. In this respect the function was independent of both the plane of preparatory irradiation and the temperature of the test. If one considers arbitrarily that only departures of 10 per cent. and more in absorption are significant, it appears that the relatively small increase of shorter radiations involved

TABLE II

RADIATION AND CARRIER EFFECTS IN ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS; VOLUME OF NUTRIENT SOLUTION 3000 CC. PLANTS SUBJECTED TO PREPARATORY NITRATE STARVATION. TEMPERATURE OF CLIMATIC CHAMBERS 15.5° ± 1.0° C. UNLESS SPECIFIED OTHERWISE, AND RELATIVE HUMIDITY ABOUT 60 PER CENT. INCREASE OF VIOLET TO ULTRAVIOLET INCLUSIVE BY USE OF THE CARBON ARC 1.0 PER CENT.

NUMBER OF TEST	DATE OF COMPLETION	PLANF OF PREPARATORY SOLAR RADIATION	ABSORPTION OF N PER GM. DRY TISSUE FROM KNO_3 UNDER COMBINED LAMPS	RELATIVE ABSORPTION BASED ON METAL CARRIER. PROPORTION WITH USE OF $NaNO_3$ AS RELATED TO KNO_3		RELATIVE ABSORPTION BASED ON SOURCE OF RADIATION. PROPORTION WITH USE OF MAZDA LAMP AS RELATED TO COMBINED LAMPS	
				UNDER MAZDA	MAZDA + ARC	WITH $NaNO_3$	KNO_3
			mg.	%	%	%	%
1	3/23/31	1200*	25	7	39	9	50
4	12/5/30†	1900	22	96	88	80	73
8	2/9/31	2540	17	71	122	74	127
9	3/11/30	2720	24	17	109	13	84
11	3/9/31	2420	17	94	80	104	90
13	4/7/30	3740	25	139	72	176	92
14	3/17/31	3920	31	102	93	91	83
15	4/22/30	4540	15	80	77	198	191
16	5/5/31†	4800	27	80	57	66	47

* Irradiated by Mazda lamp.

† Test conducted at 21.0° ± 1.0° C.

here promoted nitrate absorption. Under these conditions potassium excelled sodium as a carrier of this radical or ion. The beneficial effects upon root condition of both irradiation by the arc lamp and supplying potassium are shown in figure 2.

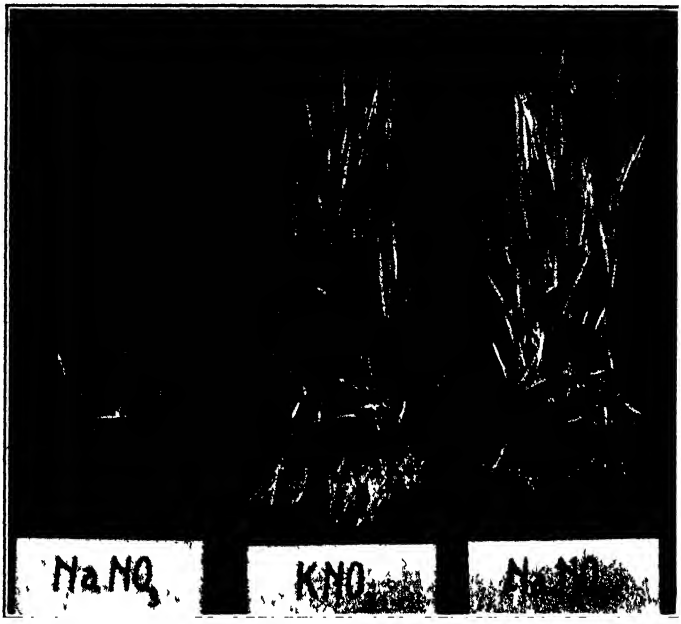


FIG. 2. Showing benefits upon root development from the increase of shorter light rays and the selection of nitrate carrier: Plants at left supplied $NaNO_3$ under the Mazda lamp only; plants at right supplied $NaNO_3$ under the combined Mazda and arc lamps; plants in center supplied KNO_3 under the combined lamps.

A summary of the results when $NaNO_3$ and KNO_3 were directly compared is presented in table III. Here are assembled the number of repetitions (in a total of eleven tests on the usual volume of nutrient solution) of departures exceeding both 5 and 10 per cent. in the absorption of nitrate. In comparing the efficiency of the radiation or carrier on the basis of a 5 per cent. departure, values between 95 and 105 per cent. have been considered as equal. Similarly, for the 10 per cent. departure values between 90 and 110 per cent. have been rated as equal. From these comparative data it appears that potassium excelled sodium as a carrier of nitrate. If the results of tests 3 and 10, which involved only the use of the combined lamps, are omitted, the superiority of potassium over sodium was essentially equal under the two forms of radiation. It is apparent also that supplementary use of the carbon arc favored the absorption of nitrate from both carriers. This superiority of potassium is in agreement

TABLE III

SUMMARY OF DEPARTURES IN ABSORPTION OF NITRATE AS RELATED TO BOTH CHARACTER OF RADIATION AND KIND OF METAL CARRIER. NINE TESTS UNDER COMBINED LAMPS AND MAZDA LAMP ALONE, TWO OTHERS UNDER COMBINED LAMPS ONLY

PERCENTAGE EXTENT OF DEPARTURE	COMPARISON OF SOURCES OF RADIATION						COMPARISON OF METAL CARRIERS					
	SUPPLYING KNO_3			SUPPLYING $NaNO_3$			UNDER MAZDA LAMP AND CARBON ARC			UNDER MAZDA LAMP ALONE		
	COMBINED LAMPS SUPERIOR	RADIATION EFFECTS EQUAL	MAZDA LAMP ALONE SUPERIOR	COMBINED LAMPS SUPERIOR	RADIATION EFFECTS EQUAL	MAZDA LAMP ALONE SUPERIOR	KNO_3 SUPERIOR TO $NaNO_3$	CARRIER EFFECTS EQUAL	$NaNO_3$ SUPERIOR TO KNO_3	KNO_3 SUPERIOR TO $NaNO_3$	CARRIER EFFECTS EQUAL	$NaNO_3$ SUPERIOR TO KNO_3
5 and above	7	0	2	6	1	2	9	0	2	6	2	1
10 and above	6	1	2	5	2	2	8	2	1	6	2	1

with the conclusion of GERICKE (3) that KNO_3 excels other nitrates when supplied to wheat in a rotation of single salts. The results suggest that increased absorption might be expected to follow raising the proportion of shorter radiations above the relatively low level of these tests.

In this connection it is of interest to note that COOPER (2) gives the energy of formation of KCl as equivalent to ultraviolet light, and suggests that an equivalent of radiant energy may be necessary for its optimal assimilation. This might imply a favorable relation of shorter rays of light to decomposition and assimilation of KNO_3. However, he places infra-red as adequate for the reduction of nitrate. NEMEC (8) found the general regions about both violet and red of the spectrum favorable to the absorption of potassium by the rye plant.

TESTS OF LIMITING FACTORS

As this investigation progressed certain tests were applied to contribute toward the elimination of limiting factors.

It was found that plants raised in the greenhouse in April to May removed per unit of dry matter about twice as much NO_3 from the complete nutrient solution at three weeks of age as compared with that removed at six weeks. The effect per unit plant was 70 per cent. as great at the earlier stage. These results indicate that the age of the plant here employed was a favorable factor.

A second test related to high metabolic requirement of potassium as possibly determinative of superiority of this element as a carrier of NO_3. Doubling the supply of KCl during the nitrate starvation period did not affect the plane of subsequent absorption from KNO_3 but depressed the intake from $NaNO_3$.

A third test was concerned with the relation of solution volume to nitrate absorption. This was actuated by the assumption that more definite evidence might follow a wider differential of NO_3 content between planted and unplanted solutions if the latter were supplied in smaller as compared with larger volumes. Approximately one-eighth the usual volume of solution was supplied in shallow dishes of the same diameter as the larger jars. The evidence supported that already presented, for in two of three trials KNO_3 exceeded $NaNO_3$ in uptake, and was absorbed to a greater extent under the combined forms of radiation than under the Mazda lamp alone. However, the plane of absorption (6 to 11 mg. N per culture) was unusually low. Stirring by slow aeration increased the absorption of KNO_3 18 per cent. and of $NaNO_3$ 12 per cent. in a greenhouse test under ordinary illumination, about one-third of the supply being removed from the stagnant solutions. In view of the free surface exposure and shallowness of these solution portions, it appears possible that the rate of renewal of salt

at the root surface was more potent than increased oxygen supply in stimulating absorption. Moreover, it should be noted that ALLISON and SHIVE[8] found aeration favorable to tissue production by soy beans only as the nutrient solution was continually renewed; yet increased aerobic respiration should be admitted as a possible favorable factor here. We purpose giving attention to this issue.

TESTS WITH COMPLETE NUTRIENT SOLUTION

The irregularities of response noted in the preceding work indicated the desirability of seeking other limiting factors. This search was directed to the use of the complete nutrient solution as well as to those of single nitrates. It included a test of omission of the nitrate starvation period, and modification of the arc lamp for the purpose of increasing the proportion of ultraviolet radiation.

Sodium nitrate formed about one-third of the complete salt mixture by weight, so that the nitrate concentration in the complete nutrient solution

TABLE IV

INFLUENCE OF PRELIMINARY STARVATION UPON SUBSEQUENT NITRATE ABSORPTION FROM A COMPLETE NUTRIENT SOLUTION BY YOUNG WHEAT PLANTS. PREPARATORY GROWTH UNDER 1200 F. C. FROM THE MAZDA LAMP AT 18°–20° C. TEST AT 15.5° ± 1° C.

PRELIMINARY NUTRIENT TREATMENT	SOURCE OF RADIATION	NITROGEN ABSORBED PER GM. DRY TISSUE PER CULTURE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION	ABSORPTION IN RELATION TO STARVATION PRACTICE
		mg.	%	%
Nitrate starved	Mazda lamp only	12.3		
		7.0		
		10.9		
		11.6		
		Ave. 10.5	100	100
Nitrate starved	Mazda + arc lamp	11.4		
		11.2		
		12.2		
		10.4		
		Ave. 11.3	108	100
Unstarved	Mazda lamp only	10.5		
		10.4		
		8.7		
		11.6		
		Ave. 10.3	100	98
Unstarved	Mazda + arc lamp	18.5		
		16.0		
		16.7		
		18.0		
		Ave. 17.3	168	153

[8] Amer. Jour. Bot. 10: 554–566. 1923.

bore that proportion to the single salt solution. The results of one test are shown in table IV, which includes the individual variability of cultures. It appears that the increase in radiation of shorter wave length was decidedly beneficial to the unstarved plants but only questionably so to those pre-starved.

Other tests were conducted under higher planes of preparatory illumination, as indicated in part by the date of completion, but with the usual plane of 1600–1680 f.c. illumination obtaining in the chamber. Unstarved plants reared on 4100 f.c. average illumination and finished on April 26 gave 20 per cent. greater nitrate absorption by supplementary use of the arc lamp. A subsequent test was completed May 15 at about 21° C. after preparatory irradiation at 4800 f.c. This gave increased absorption under supplementary use of the arc of 19 per cent. on the complete solution, 111 per cent. on KNO_3, and 51 per cent. on $NaNO_3$. In this case the absorption of nitrate per unit of dry matter from the complete solution declined appreciably (from 11–15 to 8–9 mg. N) at the higher temperature. This response may be related to the accumulation of nitrate observed earlier (**10**, table IX) at high temperature, which would tend to limit the absorption process. The higher attendant respiratory activity could be expected to decrease the plane of reducing substances in the tissue, and thus to limit both assimilation and absorption of NO_3. A test completed November 30 under 1300 f.c. average preparatory solar radiation gave an increased nitrate absorption of 25 per cent. from the complete solution under the com-

TABLE V

INFLUENCE OF PRELIMINARY NITRATE STARVATION UPON NITRATE ABSORPTION FROM A SOLUTION OF KNO_3 BY YOUNG WHEAT PLANT. PREPARATORY GROWTH UNDER 2000 F.C. RADIATION FROM THE MAZDA LAMP

SOURCE OF RADIATION	PRELIMINARY NUTRIENT TREATMENT	WEIGHT OF ENTIRE DRIED PLANT	NITROGEN ABSORBED PER GM. DRY TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION (AVERAGE)	ABSORPTION IN RELATION TO STARVATION PRACTICE (AVERAGE)
		gm.	*mg.*	%	%
Mazda lamp only	Starved	5.3 5.6	14.0 14.0	100	100
Mazda and arc	Starved	5.7 5.4	14.2 14.6	103	100
Mazda lamp only	Unstarved	5.7 6.0	15.1 15.9	100	111
Mazda and arc	Unstarved	5.9 6.1	18.1 16.2	111	120

bined lamps. The absolute amounts of nitrogen absorbed from $NaNO_3$ and KNO_3 were of the same order as from the complete solution, and increased 18 and 20 per cent. respectively by supplementary use of the carbon arc.

A further test of the influence of nitrate starvation upon subsequent absorption was made with a nitrate alone. The results are given in table V, wherein the weight of dried plants is included to show the extent of variation of this factor. From these data it is further evident that preliminary starvation in respect to NO_3 was an unfavorable feature of our earlier tests. The plane of absorption from a complete solution was also favorable in unstarved plants, as shown in table VI.

TABLE VI

INFLUENCE OF INCREASED PROPORTIONS OF SHORTER RADIATIONS UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS WITHOUT PRELIMINARY NITRATE STARVATION. AVERAGE PLANE OF SOLAR RADIATION IN PREPARATORY GROWTH PERIOD 1540 F.C. COMPLETED DECEMBER 16, 1931

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITROGEN CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO RADIATION (AVERAGE)
		mg.	*gm.*	*mg.*	%
Mazda lamp only	Complete solution	122	6.6	19.1	100
		122	6.3	19.5	
	KNO_3	124	5.6	15.4	100
	$NaNO_3$	125	5.9	16.5	100
Mazda and arc	Complete solution	122	5.7	22.2	115
		122	5.5	22.2	
	KNO_3	124	5.6	18.7	121
	$NaNO_3$	125	6.1	18.9	115

IMPROVEMENT OF CARBON ARC

Increased amperage results in an increase of short wave emission by the carbon arc, as shown by COBLENTZ and associates (1). With the aid of the Service Department of this institution our lamps were reconstructed to give this effect. A part of the current was shunted around the lamp resistance and the entire unit was operated through an appropriate rheostat. Current consumption was thus converted from a value of 7.5 amperes at 110 volts to 22 amperes at 45 volts, across the arc. The reconstructed lamp was equipped with impregnated carbons of the Sunshine type, 13 mm. in diameter. An examination in the manner already indicated of the intensity and distribution of radiation from the altered lamp included the use of an additional glass screen (G586 A, with transmission coefficients given by

GIBSON and associates **4**, fig. 21, curve 83). This transmits strongly in the longer ultraviolet close to the maximum of emission from the arc, and to a slight extent in the red region. The total intensity of the arc from the modified lamp was about 50 per cent. greater than that of the form used earlier, and the same relation held for the regions defined in table I. In the latter regions, therefore, the radiation effects had not been appreciably increased. However, the emission from the newer form of arc transmitted by screen G586 A, which was essentially solar ultraviolet, was more than twice as intense as that from the older form. With the increased time of operation the reconstructed lamp increased the proportion of this spectral region alone by somewhat less than 1 per cent. of the total radiation emitted by the Mazda lamps, equivalent to an increase of over 5 per cent. in the region specified.

Two series of cultures were conducted with use of the reconstructed arc lamp, the time of differential radiation being two daily periods of six hours each, without preliminary starvation. The data appear in tables VII and VIII, and indicate that the increased proportion of long ultraviolet rays considerably favored the absorption of nitrate. The beneficial effect of a continuous supply of nitrate is evident also in the relatively high planes of weight of nitrogen absorbed.

TABLE VII

INFLUENCE OF IMPROVED CARBON ARC UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS. AVERAGE PLANE OF SOLAR RADIATION DURING PREPARATORY GROWTH 3410 F.C. TEST COMPLETED APRIL 6, 1932

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITRATE CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION
Mazda lamp only	$NaNO_3$	314	6.1	22.1	100
	$NaNO_3$	314	6.0	23.2	
	KNO_3	317	5.4	24.0	100
	KNO_3	317	5.8	23.6	
Mazda + Sunshine carbon arc	$NaNO_3$	314	5.5	28.1	124
	$NaNO_3$	314	5.6	28.2	
	KNO_3	317	5.8	30.1	127
	KNO_3	317	5.6	30.2	

The nearest approach to a comparison of the forms of radiation here applied with sunlight appears to be offered by the data of COBLENTZ and associates (**1**, table II). For the proportion between the region 3500 to 4500 Å and the total emission from 3500 to 6000 Å, these give the following percentages: June sunlight 38, tungsten lamp (115 V. a.c., 12.7 amp.) 21, carbon arc (50 V. a.c., 5 amp.) 53, and white flame arc (70 V. a.c., 20 amp.)

TABLE VIII

INFLUENCE OF IMPROVED CARBON ARC UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS. AVERAGE PLANE OF SOLAR RADIATION DURING PREPARATORY GROWTH 4050 F.C. TEST COMPLETED MAY 8, 1932

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITROGEN CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION (AVERAGE)
		mg.	*gm.*	*mg.*	%
Mazda lamp only	Complete solution	124	5.4	25.1	100
		124	5.7	24.8	
	KNO_3	322	5.5	21.5	100
	$Ca(NO_3)_2$	311	5.9	23.0	100
	95 mols KNO_3 + 5 mols $Ca(NO_3)_2$	315	5.3	25.0	100
Mazda + Sunshine carbon arc	Complete solution	124	6.1	34.1	136
		124	5.5	33.9	
	KNO_3	322	5.9	26.1	122
	$Ca(NO_3)_2$	311	6.0	30.0	130
	95 mols KNO_3 and 5 mols $Ca(NO_3)_2$	315	5.7	29.0	116

60. An attempt to apply these values to our experiment gave only approximately 23 per cent. of shorter radiations in our most efficient system. In view of the relative composition of sunlight this is little improvement over exposure to the Mazda lamp only. COBLENTZ and associates (**1**, fig. 18) give a graphic comparison of the general distribution of radiation from the sun and the carbon arc, and LUCKIESCH (**7**) shows the curve of distribution from an ordinary 500-watt Mazda lamp. From these the deficiency of the arc lamp as a means of supplementing the emission of the Mazda lamp in the blueviolet region becomes apparent.

Summary

1. Following preparatory nitrate starvation, Progress wheat plants approaching three weeks of age were exposed to about 1600 f.c. illumination at about 15.5° C. while supplied single nitrates. Under these conditions the NO_3 radicle was absorbed more freely from KNO_3 in the nutrient solution than from the nitrates of other common metals. This absorption was promoted by increases in blue to longer ultraviolet radiations above those emitted by the common Mazda lamp.

2. As here produced and tested, both with single nitrates and with a complete solution, young wheat plants responded to an increase in the proportion of blue to ultraviolet light more decidedly without preliminary

nitrate starvation than when so prepared. With KNO_3 this response was apparently not due to deficiency of potassium in the starved plants.

3. A substantial further increase in the proportion of long ultraviolet radiation promoted the absorption of nitrate somewhat further. Rough measurements of the distribution of radiation here effective indicate that it was decidedly deficient in other shorter radiations as compared with sunlight.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. COBLENTZ, W. W., DORCAS, M. J., and HUGHES, C. W. Radiometric measurements of the carbon arc and other light sources used in phototherapy. Scientific Paper 539. Bur. Standards, U. S. Dept. Commerce. 1926. Fig. 4.
2. COOPER, H. P. Ash constituents of pasture grasses, their standard electrode potentials and ecological significance. Plant Physiol. **5**: 193–214. 1930.
3. GERICKE, W. F. On the physiological balance in nutrient solutions for plant cultures. Amer. Jour. Bot. **9**: 180–182. 1922.
4. GIBSON, K. S., TYNDALL, G. P. T., and MCNICHOLAS, H. J. The ultraviolet and visible transmission of various colored glasses. Technological Paper 148. Bur. Standards, U. S. Dept. Commerce. 1920. Fig. 21, curve 83.
5. HARPER, H. J. The accurate determination of nitrates in soils. Jour. Ind. and Eng. Chem. **16**: 180–183. 1924.
6. LIVINGSTON, B. E. (Editor). A plan for cooperative research on the salt requirements of representative agricultural plants. 2d ed. Baltimore. 1919.
7. LUCKIESCH, M. Artificial sunlight. New York. 1930. Fig. 27.
8. NEMEC, ANTONIN, and MIHOVIL, GRACANIN. Influence de la lumière sur l'absorption de l'acide phosphorique et du potassium par les plantes. Compt. Rend. Acad. Sci. Paris **182**: 806–808. 1926.
9. POPP, H. W. A physiological study of the effect of light of various ranges of wave lengths on the growth of plants. Amer. Jour. Bot. **13**: 706–736. 1926.
10. TOTTINGHAM, W. E. Temperature effects in the metabolism of wheat. Plant Physiol. **1**: 307–336. 1926. p. 311.
11. ————————, and LOWSMA, HENRY. Effects of light upon nitrate assimilation in wheat. Jour. Amer. Chem. Soc. **50**: 2436–2445. 1928.
12. WARBURG, O., and NEGELEIN, E. Über die Reduktion der Salpetersäure in grünen Zellen. Biochem. Zeitschr. **110**: 66–115. 1920.

AMOUNTS OF BOUND AND FREE WATER IN AN ORGANIC COLLOID AT DIFFERENT DEGREES OF HYDRATION[1]

HELEN L. CHRYSLER

(WITH TWO FIGURES)

Introduction

All of the water in a colloidal system does not possess the same physical properties. This is shown by the fact that when the vapor pressure of the water in such systems is reduced, all of the water does not act as a solvent and some of it cannot be removed by pressure or by freezing. The water in the system which is free to exert those properties characteristic of the bulk of the water in dilute solutions has been termed free water in contrast to that part of the water which is bound or held by the colloid.

It is believed that the ability of the cell colloids to bind water has an important significance in certain physiological processes. SHULL (**27**) measured the forces with which the colloidal gels of the seed attract water by putting the seeds in solutions of very high known osmotic concentrations. He found that an air-dry seed of *Xanthium* with 7 per cent. hygroscopic moisture showed an initial imbibition pressure of about 1000 atmospheres.

Cold resistance has been considered by LOTT (**13**), MARTIN (**14**), NEWTON (**19, 20**), ROBINSON (**23**), ROSA (**24**), and others to be related to the amount of hydrophilic colloids present in the tissue fluids. All of these workers believe that the hardier the species the more hydrophilic colloids are present and the greater the imbibition pressure of the tissues. It has been observed that this correlation does not exist when the material is in the unhardened condition. MEYER (**16**), studying cold resistance of the leaves of the pitch pine, obtained results which he regarded as evidence of this same relationship. His later data (**17**), however, seem to show that the binding of water by colloids is not an important factor in the cold resistance of this species.

NEWTON and MARTIN (**22**), in studying the drought resistance of a number of cereals, grasses, and other plants, concluded that the imbibition pressure of the hydrophilic colloids in the plant tissue fluids is the most significant factor in retaining moisture, and that drought resistant varieties can be differentiated from non-resistant varieties by bound water content. FRITSCH and HAINES (**5**) observed that cells of terrestrial algae surviving after a long period of drought have more or less highly viscous protoplasts without any large vacuoles. This was also the normal condition of the more drought resistant species. They consider that this gel condition

[1] Papers from the Department of Botany, the Ohio State University, no. 305.

of the protoplast may indicate that imbibition phenomena are principally concerned in the drought resistance of these plants.

BAILEY and GURJAR (1) have investigated the relation of moisture content to respiration in stored wheat. The rate of respiration increased very gradually with increase in the moisture content from 12.5 to 14.78 per cent. Above a moisture content of 14.78 per cent. the rate was greatly accelerated. It has been suggested by GORTNER (6) that this sharp break in the respiration curve may indicate that below 14.78 per cent. moisture, all or most of the water was in the bound condition, and that the rapid increase in the respiration rate was related to an increase in the amount of free water present.

WHITCOMB and SHARP (31) studied the germination of frozen and unfrozen wheat that had been harvested at different stages of maturity. They found that at a moisture content of 56.2 per cent. or more the seeds were almost invariably killed by freezing at a temperature of –20° to –28° C. for 48 hours. However, when seeds with a moisture content of 50.6 per cent. or less were treated in the same way, there was a great increase in the percentage of germination, the percentage germinating ranging as high as 98 per cent. GORTNER (6) suggests that an explanation for this may also be found in the free-bound water equilibrium of the seeds. The data of KIESSELBACH and RATCLIFF (12), on the relative germination of seed corn of varying moisture content after exposure to low temperatures, likewise show that death from freezing is directly related to the moisture content of the grain and the temperature of exposure.

These illustrations indicate that the condition of the water in a biological system has a marked effect on the properties of the protoplasm, and give evidence in support of the supposition that bound water is closely related to the physiological processes carried on by living cells.

It was the purpose of this investigation to determine as nearly as possible the amounts of bound and free water in an organic colloid which had been allowed to imbibe various percentages of water while other conditions were kept constant. A study of the relation of the concentration of dry matter in a colloidal system to the free-bound water ratio, over a wide range of hydrations comparable with those found in protoplasm, should give information of some value for comparison with the complex colloidal system in living cells, and may throw some light on the part played by colloids and bound water in certain physiological processes. At the time this work was begun, no studies of this sort had been reported. Recently, however, several investigators (2, 11, 22) have published reports of similar studies with other types of colloidal systems. These papers will be referred to in more detail in the discussion.

Methods and material

The material chosen for use in this investigation was the stipe of the Elk kelp, *Pelagophycus porra* (Leman) Setchell. This was selected because it is an excellent example of an organic substance possessing great hydration capacity. The structural components of the apparently non-vacuolate cells of this alga are probably largely carbohydrate gels such as gums and mucilages, which yield both hexoses and pentoses on hydrolysis. These substances are highly hydrophilic.

For the purpose of this investigation, all the kelp stipe was prepared at one time as follows. The sun-dried stipe as received at the laboratory was soaked in tap water until maximum swelling was attained. During this period the water was changed several times each day. Only those pieces that showed the greatest degree of swelling were selected for use in the investigation. These pieces of swollen stipe were in the form of hollow cylindrical tubes 2–3 inches in diameter with walls about 0.25 inch thick. The outer epidermis was scraped off the selected pieces to aid in further washing and to make the pieces as uniform as possible. Next the swollen stipe was cut into pieces approximately 0.5 inch long, 0.25 inch wide, and 0.25 inch in thickness. These pieces were washed in running tap water for 24 hours, thoroughly washed through a number of changes of distilled water, and finally spread out in boxes in the sun to dry. After about three days the material was air-dry. It was then placed in desiccators over fresh calcium chloride to complete the drying process and left until used in the determinations. An analysis of the washed kelp showed the percentage of ash to be about 0.09 per cent.

The free water was determined by the calorimeter or heat-of-fusion method. This method was first introduced by MÜLLER-THURGAU (**18**) and later modified by RUBNER (**25**) and THOENES (**29**). Certain improvements have recently been made in the method by ROBINSON (**23**), ST. JOHN (**28**), SAYRE (**26**), and MEYER (**17**). The equation used in calculating the results of this investigation is also the one developed by these investigators. According to this method the material is cooled to –20° C. and a determination made of the amount of water which is frozen at the end of the cooling period. This method assumes that at –20° C. all of the free water will be converted into ice and that the free-bound water equilibrium is not altered by the freezing. The value for free water is subtracted from the total water and the remainder is considered to be bound water.

The apparatus and procedure used in making the free water, correction factor, and specific heat determinations are in general the same as those described by MEYER (**17**). It will be necessary, therefore, to describe here only the points in which the methods used in this investigation differ from those described in that paper.

In preparing the dried kelp for the determinations, it was taken from the desiccator, weighed, and put in distilled water long enough to reach approximately the desired hydration. It was then taken from the water, wiped off, weighed again, and the percentage hydration calculated. The temperature was kept constant by keeping the beaker containing the kelp and distilled water in a 30° C. oven except when weighings were being made. Preliminary experiments had been made to determine how long it took the dried kelp to imbibe various percentages of water, and how much dry material was needed for determinations at various hydrations, and a working chart prepared from these data.

The hydration percentages used in these determinations ranged from 30 to 1015 per cent. The degree of hydration is expressed as the percentage of water absorbed by the dry kelp. At a hydration percentage of 100, each gram of dry kelp had imbibed one gram of water. In order to make the conditions as uniform as possible for the samples of kelp at the various hydrations, the same time interval was allowed from the time the desiccator-dried kelp was put in distilled water and the time the samples were put in the freezing bath, regardless of the percentage of hydration. This interval was 16 hours, and was based on the time required to reach maximum hydration in preliminary tests.

Water was used as the calorimeter liquid in preliminary tests, but it was soon discovered that heat produced by the imbibition of water by the kelp gave erroneous temperature readings. Benzene was then selected, because it is not appreciably imbibed by the kelp and because its density is low enough so that the material, even when dry, was well immersed in it. Exactly 220 grams of pure benzene with a volume of 250 cc. at 20° C. were used in making each calorimetric determination. A fresh supply of benzene was used for each test.

Fifteen-gram samples of hydrated kelp were used except for the two highest hydrations, when the weight of the sample was reduced to 10 grams. This was necessary because the range of temperature on the calorimeter thermometer was not great enough to measure the lowest temperature of the benzene if 15-gram samples were used.

In all of the determinations reported in this paper, the system was brought to equilibrium by stirring the contents of the calorimeter with a stirring rod made of a loop of iron wire of small diameter. A hole large enough to admit the wire was provided in the stopper of the calorimeter. The time required to reach equilibrium was about 5 minutes.

Calculation of the amount of water which freezes within the material was made according to the following equation. An explanation of the derivation of this equation may be found in the paper by MEYER (**17**).

$$W_f = \frac{FNS(T-T_e) - [W_dS_d(T_e-T_s) + W_wS_w(T_e-T_s)]}{Q - [(S_b-S_l)(T_m-T_s)]}$$

Where

F = Correction factor.
N = Weight of benzene in grams.
S = Mean specific heat of benzene for temperature range $T-T_e$.
T = Temperature of benzene at beginning of test.
T_e = Equilibrium temperature of benzene.
W_d = Weight of dry matter in the sample in grams.
S_d = Mean specific heat of dry kelp for the temperature range T_e-T_s.
T_s = Temperature of the sample at beginning of test.
W_w = Weight of all the water in the sample in grams.
S_w = Mean specific heat of all water in the sample for temperature range T_e-T_m.
S_b = Mean specific heat of unfrozen water for the temperature range T_m-T_s.
T_m = Melting point of ice in the sample = freezing temperature of free water.
S_l = Mean specific heat of ice in the sample for the temperature range T_m-T_s.
Q = Heat of fusion of ice at T_m.

It is necessary to determine a correction factor for the calorimeter, as the heat absorbed by the sample is taken not only from the benzene but also from the walls of the calorimeter and the thermometer and stirrer in contact with the benzene. The value obtained for free water is too low unless this correction is made. The value for the correction factor was taken as 1.05 for a 15-gram sample, this being the average value obtained from twenty closely agreeing determinations. The correction factor for a 10-gram sample would be slightly lower than this. The same value was used, however, as other experiments have shown that the error involved was so small that it could be disregarded without any appreciable effect on the results.

The average value determined for the specific heat of dry kelp was 0.27 calories. This is probably a minimum value, as the recorded values for the specific heat of other substances of similar chemical composition are somewhat higher. The values used for the specific heat of pure benzene were secured by plotting a curve for values as given in the International Critical Tables (**30**). The values for the specific heat of ice and heat of fusion of ice were obtained from tables by DICKINSON and OSBORNE (**3**). The values for the specific heat of water above 0° C. are those given in the Handbook of Physics and Chemistry (**10**). The values for the specific heat of water from 0° to −20° C. were obtained by extrapolating the curve plotted for the values above 0° C. given in the table just mentioned. It has been as-

sumed that the specific heat of bound water is the same as that of free water at the same temperature.

Results and discussion

A series of three calorimetric determinations was made for each hydration percentage. These values and the average values for each hydration are presented in table I. Figure 1 gives curves for the average amounts

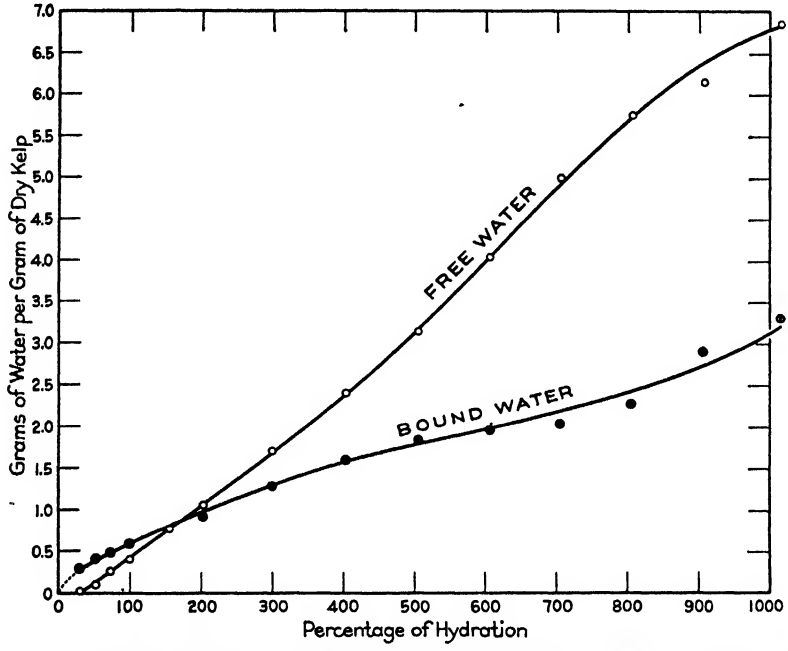


FIG. 1. Amounts of bound and free water in kelp stipe at different percentages of hydration.

of bound and free water per gram of dry kelp at the different percentages of hydration. The amounts of bound and free water per gram of dry kelp are plotted as ordinates against the percentages of hydration as abscissas.

These data show that the amount of imbibed water bound per gram of dry kelp increases with an increase in hydration, but not proportionately; hence the percentage of imbibed water bound decreases with increased hydration. At the lowest hydration of 30 per cent., 0.283 gram of water (95.83 per cent.) was bound per gram of dry material. As the hydration was increased the amounts of both bound and free water per gram of dry material showed consistent increases, the bound water at first increasing more rapidly than the free water, until a hydration of 156.6 per cent. was reached. Here the amounts of bound and free water were approximately equal. Above this hydration the amount of free water per gram of dry

TABLE I

FREE AND BOUND WATER IN KELP STIPE AT DIFFERENT DEGREES OF HYDRATION

HYDRATION PER GM. DRY KELP	DRY KELP	FREE WATER	BOUND WATER	FREE WATER PER GM. DRY KELP	BOUND WATER PER GM. DRY KELP	BOUND WATER AS PERCENTAGE IMBIBED WATER
%	%	*gm.*	*gm.*	*gm.*	*gm.*	%
30.00	76.92	0.100	3.312	0.009	0.287	97.07
		0.095	3.317	0.008	0.287	97.22
		0.232	3.180	0.020	0.276	93.20
Average		0.142	3.270	0.012	0.283	95.83
50.91	66.27	0.940	4.021	0.094	0.404	81.05
		0.851	4.110	0.086	0.415	82.85
		1.043	3.918	0.105	0.394	78.98
Average		0.945	4.016	0.095	0.404	80.96
73.94	57.49	2.212	4.064	0.256	0.471	64.75
		2.157	4.119	0.250	0.478	65.63
		2.005	4.271	0.232	0.495	68.05
Average		2.125	4.151	0.246	0.481	66.14
100.00	50.00	3.197	4.253	0.426	0.567	57.09
		2.932	4.518	0.391	0.602	60.64
		3.098	4.252	0.413	0.567	57.85
Average		3.075	4.341	0.410	0.579	58.53
136.60	39.03	4.498	4.597	0.768	0.785	50.54
		4.449	4.646	0.760	0.794	51.08
		4.563	4.532	0.779	0.774	49.83
Average		4.503	4.592	0.769	0.784	50.48
201.90	33.33	5.466	4.334	1.093	0.867	44.22
		5.527	4.273	1.106	0.855	43.60
		4.822	4.978	0.964	0.996	50.80
Average		5.272	4.528	1.054	0.906	46.20
300.00	25.00	6.072	5.078	1.619	1.354	45.54
		6.382	4.768	1.702	1.271	42.76
		6.589	4.561	1.757	1.216	40.91
Average		6.348	4.802	1.693	1.280	43.07
401.60	19.93	7.002	4.959	2.342	1.659	41.46
		7.447	4.514	2.498	1.510	37.74
		7.198	4.763	2.408	1.593	39.82
Average		7.216	4.745	2.416	1.587	39.67
505.60	16.51	7.411	4.912	2.991	1.983	39.86
		8.249	4.074	3.330	1.645	33.06
		7.625	4.698	3.078	1.897	38.12
Average		7.762	4.561	3.133	1.842	37.01
605.00	14.19	8.307	4.516	3.905	2.123	35.22
		8.186	4.637	3.849	2.180	36.16
		9.431	3.392	4.434	1.595	26.45
Average		8.641	4.182	4.063	1.966	32.61
704.40	12.43	9.183	3.952	4.925	2.114	30.09
		8.770	4.365	4.703	2.341	33.23
		10.019	3.116	5.373	1.671	23.72
Average		9.324	3.811	5.000	2.042	29.01
804.40	11.06	9.259	4.083	5.584	2.463	30.60
		10.263	3.079	6.190	1.857	23.08
		9.106	4.236	5.492	2.535	31.75
Average		9.543	3.799	5.755	2.292	28.48
906.60	9.40	9.292	4.218	6.236	2.831	31.22
		9.148	4.362	6.139	2.927	32.29
		9.081	4.429	6.094	2.973	32.78
Average		9.174	4.336	6.156	2.910	32.09
1015.00	8.97	9.552	4.102	7.100	3.049	30.04
		9.175	4.479	6.820	3.329	32.80
		8.913	4.741	6.625	3.537	34.72
Average		9.213	4.441	6.848	3.305	32.52

material showed a more rapid increase than the amount of bound water per gram of dry material, but both showed continuous increases until a hydration of 1015 per cent. was reached. This represents nearly the maximum hydration for this material. At this hydration 3.305 grams of water (32.52 per cent.) were bound per gram of dry kelp. These values indicate that the proportion of dry material in a colloidal system is a factor which greatly affects the weight of water bound per gram of dry material.

FREUNDLICH (4) has proposed a general parabolic equation for an adsorption reaction. The curve for bound water given in figure 1 appears to approximate a parabola. When these same values are converted into logarithms and graphically plotted, the points tend to fall on a straight line as shown in figure 2. The values of any parabolic equation plotted as

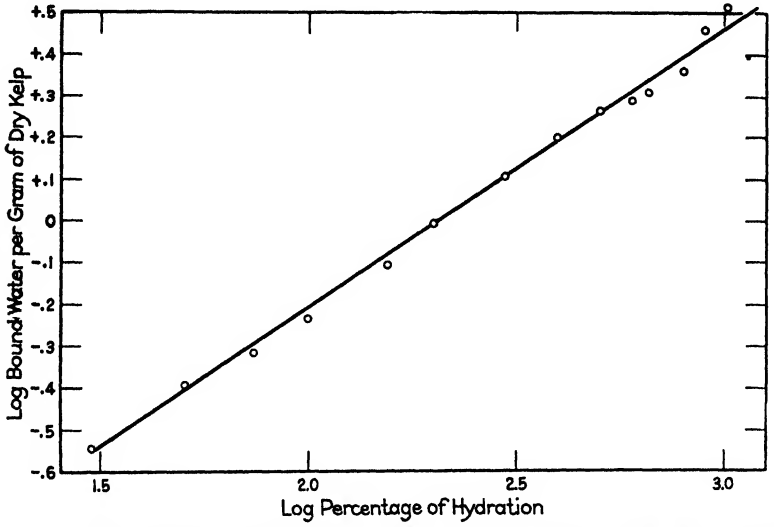


FIG. 2. Bound water and percentage of hydration values of figure 1 expressed logarithmically.

logarithms give a straight line curve. This indicates that water binding in kelp stipe may be considered as an adsorption phenomenon.

The same general type of curve for the relation between the concentration of dry material in a colloidal system and bound water was obtained in these determinations as has been reported recently by BRIGGS (2), JONES and GORTNER (11), and NEWTON and MARTIN (22). These studies, however, except for the calorimetric data of BRIGGS, were all made over a much narrower range of water content than were the results reported here. It is necessary to have at hand data covering the whole range of hydration of a colloid in order to gain a complete idea of the relationship between the dry material and the condition of the water in the system. This is espe-

cially true if comparisons are to be made with the colloidal systems of living cells where the range in possible moisture contents is very wide.

When bound water values for different systems of the same concentration of dry material are compared, the values usually vary considerably. This difference in the absolute values determined for bound water may be due to the dissimilarity in the nature of the colloidal systems used, or to the fact that different methods were used in obtaining the values. That the type of colloidal system is a factor in affecting the degree of water binding has been shown by several of the previous investigators. This study reports comparatively high bound water values for kelp stipe. NEWTON and MARTIN (**22**) and BRIGGS (**2**) both obtained their highest values for bound water with agar, a carbohydrate material similar in composition to kelp stipe, which may be taken as further evidence that kelp stipe has a relatively high water-binding capacity.

SAYRE (**26**) made a comparison of three methods of measuring bound water in an 18.6 per cent. gum arabic solution. He found excellent agreement in the mean values for bound water as determined by the three methods. However, when the same methods were applied on the same sample of expressed sap from corn tissues, the results were not in such good agreement. BRIGGS (**2**) determined the relative vapor pressure-water content curve for a purified colloid and then compared values for bound water obtained by different methods with points on this curve. He found that the cryoscopic method of NEWTON and GORTNER (**21**) and the vapor pressure method of HILL (**9**) yielded to a satisfactory explanation in terms of this curve, but he could not explain the different results obtained when the calorimeter method was used. GORTNER (**7**) emphasizes the fact that there is no sharp division line between bound and free water in a colloidal system. He suggests that this might be the reason why results obtained by different methods may vary so widely, since one method may measure the water at one degree of activity and another method at a slightly different degree of activity.

Attempts have been made to discover whether any correlations exist between bound water and other properties of living tissues. As previously noted, a number of investigators have observed a positive correlation between bound water, hydrophilic colloids, and cold resistance. Other factors, as sugar and moisture contents of the tissue and osmotic pressure and total solids of the tissue fluids, have also been observed by some of these workers to be related to bound water.

NEWTON and MARTIN (**22**) reported a high positive correlation between percentage bound water, concentration of solids, and osmotic pressure in the tissue fluids of some thirteen species of grasses of different degrees of drought resistance.

GORTNER and RUDE (8) calculated the coefficients of correlation for all the various combinations possible given in the data of MEYER (15) on the physico-chemical properties of plant saps from some fifty species of mesophytic plants of Ohio. The properties which had been determined were percentage water and yield of sap from the leaves; and of the total solids, osmotic value and percentage bound water of the expressed leaf saps. They did not find high coefficients of correlation between bound water and any of the other factors.

The recent work of MEYER (17) on cold resistance in the leaves of the pitch pine shows a positive correlation between the total hydration of the tissues and the amounts of both bound and free water per gram of dry material. These data are in accord with the results reported here concerning the relation of bound water to the hydration of the tissues of kelp.

The ranges of hydration of the kelp stipe as used in these experiments correspond in general to the ranges of hydration of such organic colloids as those found in living protoplasm, cell walls, etc. A hydration of 1015 per cent., the maximum attained in these determinations, represents approximately a 91 per cent. water content. The hydration of colloids occurring in plant cells may vary over a wide range but does not greatly exceed the range studied for kelp stipe in this investigation. The data obtained, therefore, may be of significance with regard to the relative amounts of bound and free water in the colloidal systems occurring in plant cells.

Summary

1. Determinations were made of the amounts of bound and free water in the stipe of the Elk kelp, *Pelagophycus porra* (Leman) Setchell, at hydrations varying from 30 to 1015 per cent. The degree of hydration is expressed as the percentage of water absorbed by the dry kelp.

2. The calorimeter or heat-of-fusion method was used in determining the amounts of bound and free water. The material was cooled to −20° C. and determinations made of the amount of water which had frozen. It is the assumption of this method that at this temperature all of the free water will be converted into ice and that the free-bound water equilibrium is not altered by the freezing.

3. The data show that the amount of imbibed water bound per gram of dry kelp increases with an increase in hydration, but not proportionately, so that the percentage of imbibed water bound decreases with increased hydration. At the lowest hydration of 30 per cent., 0.283 gram of water (95.83 per cent.) was bound per gram of dry material. As the hydration was increased the amounts of both bound and free water per gram of dry material showed consistent increases, the bound water at first increasing more rapidly than the free water, until a hydration of 156.6 per cent. was

reached. Here the amounts of bound and free water were approximately equal. Above this hydration the amount of free water per gram of dry material showed a more rapid increase than the amount of bound water per gram of dry material, but both showed continuous increases until a hydration of 1015 per cent. was reached. This represents nearly the maximum hydration for this material. At this hydration 3.305 grams of water (32.52 per cent.) were bound per gram of dry kelp. These values indicate that the proportion of dry material in a colloidal system is a factor which greatly affects the weight of water bound per gram of dry material.

4. The same general type of curve for the relation between the concentration of dry matter in a colloidal system and bound water was obtained in these determinations as has been reported recently by several other investigators for other kinds of colloidal systems, although in general they worked with a much narrower range of hydration.

5. The ranges of hydration of the kelp stipe as used in these experiments correspond closely to the ranges of hydration of such organic colloids as those found in living protoplasm, cell walls, etc. The data obtained, therefore, may be of significance with regard to the relative amounts of bound and free water in such systems.

The writer desires to express appreciation to Dr. B. S. MEYER of the Department of Botany, who suggested this problem and gave many helpful suggestions and criticisms during the progress of this investigation and the preparation of this paper.

OHIO STATE UNIVERSITY
COLUMBUS, OHIO

LITERATURE CITED

1. BAILEY, C. H., and GURJAR, A. M. Respiration of stored wheat. Jour. Agr. Res. **12**: 685–713. 1918.
2. BRIGGS, D. R. Water relationships in colloids. II. "Bound" water in colloids. Jour. Phys. Chem. **36**: 367–386. 1932.
3. DICKINSON, H. C., and OSBORNE, N. S. Specific heat and heat of fusion of ice. Bur. Standards Bull. 12. 49–81. 1915.
4. FREUNDLICH, H. Colloid and capillary chemistry. Translated by H. S. HATFIELD. Methuen and Co. Ltd. London. 1926.
5. FRITSCH, F. E., and HAINES, F. M. The moisture relations of terrestrial algae. II. The changes during exposure to drought and treatment with hypertonic solutions. Ann. Bot. **37**: 683–728. 1923.
6. GORTNER, R. A. Outlines of biochemistry. John Wiley and Sons. New York. 1929.

7. ————————. The state of water in colloidal and living systems. Trans. Faraday Soc. **26**: 678–686. 1930.
8. ————————, and RUDE, RACHEL. Interrelationships of certain physico-chemical constants of plant saps. Proc. Soc. Exp. Biol. Med. **25**: 630–635. 1928.
9. HILL, A. V. The state of water in muscle and blood and the osmotic behavior of muscle. Proc. Roy. Soc. **106B**: 477–505. 1930.
10. HODGMAN, C. D., and LANGE, N. A., editors. Handbook of chemistry and physics. Ed. 16. Chemical Rubber Pub. Co. Cleveland. 1931.
11. JONES, I. D., and GORTNER, R. A. Free and bound water in elastic and non-elastic gels. Jour. Phys. Chem. **36**: 387–436. 1932.
12. KIESSELBACH, T. A., and RATCLIFF, J. A. Freezing injury of seed corn. Nebraska Agr. Exp. Sta. Res. Bull. 16. 1920.
13. LOTT, R. V. Correlation of chemical composition with hardiness in brambles. Missouri Agr. Exp. Sta. Res. Bull. 95. 1926.
14. MARTIN, J. H. Comparative studies of winter hardiness in wheat. Jour. Agr. Res. **35**: 493–535. 1927.
15. MEYER, B. S. Studies of physical properties of leaves and leaf saps. Ohio Jour. Sci. **27**: 263–288. 1927.
16. ————————. Seasonal variations in the physical and chemical properties of the leaves of the pitch pine, with especial reference to cold resistance. Amer. Jour. Bot. **15**: 449–472. 1928.
17. ————————. Further studies on cold resistance in evergreens, with special reference to the possible rôle of bound water. Bot. Gaz. **94**: 297–321. 1932.
18. MÜLLER-THURGAU, H. Über das Gefrieren und Erfrieren der Pflanzen Landw. Jahrb. **9**: 133–189. 1880; **15**: 453–610. 1886.
19. NEWTON, R. The nature and practical measurement of frost resistance in winter wheat. Univ. Alberta Col. Agr. Res. Bull. 1. 1924.
20. ————————. Colloidal properties of winter wheat plants in relation to frost resistance. Jour. Agr. Sci. **14**: 178–191. 1924.
21. ————————, and GORTNER, R. A. A method for estimating hydrophilic colloid content of expressed plant tissue fluids. Bot. Gaz. **74**: 442–446. 1922.
22. ————————, and MARTIN, W. M. Physico-chemical studies on the nature of drought resistance in crop plants. Canadian Jour. Res. **3**: 336–427. 1930.
23. ROBINSON, W. Relation of hydrophilic colloids to winter hardiness of insects. Colloid Symposium Monograph **5**: 199–218. 1928.
24. ROSA, J. T. Investigations on the hardening process in vegetable plants. Missouri Agr. Exp. Sta. Res. Bull. 48. 1921.

25. RUBNER, M. Über die Wasserbindung in Kolloiden mit besonderer Berücksichtigung des quergestreiften Muskels. Abh. preuss. Akad. Wiss., phys. math. Klasse. 3–70. 1922.
26. SAYRE, J. D. Methods of determining bound water in plant tissue. Jour. Agr. Res. **44**: 669–688. 1932.
27. SHULL, C. A. Semipermeability of seed coats. Bot. Gaz. **56**: 169–199. 1913.
28. ST. JOHN, J. L. The temperature at which unbound water is completely frozen in a biocolloid. Jour. Amer. Chem. Soc. **53**: 4014–4019. 1931.
29. THOENES, F. Untersuchungen zur Frage der Wasserbindung in Kolloiden und tierischen Geweben. Biochem. Zeitschr. **157**: 174–186. 1925.
30. WASHBURN, E. W., editor. International critical tables of numerical data, physics, chemistry, and technology. 1st ed. vol. V. McGraw-Hill Book Co., Inc. New York and London. 1929.
31. WHITCOMB, W. O., and SHARP, P. F. Germination of frozen and non-frozen wheat harvested at various stages of maturity. Jour. Agr. Res. **31**: 1179–1188. 1925.

GRADIENT COMPOSITION OF ROSE SHOOTS FROM TIP TO BASE[1]

H. B. TUKEY AND E. L. GREEN

(WITH SIX FIGURES)

Introduction

In the commercial propagation of plants by means of stem cuttings, it is customary to remove a shoot from the plant and divide it into sections, or "cuttings," which are then placed in the rooting medium. It is common knowledge that the tip cutting from a shoot is less woody than one taken from the middle portion, and that a cutting from the basal portion is in turn still more woody, indicating that the structure and degree of differentiation vary from tip to base.

Moreover, the behavior of cuttings, as regards their rooting responses, varies with the degree of "hardness." SCHRADER (3) has shown for the tomato, and ZIMMERMAN (4) for *Weigela* and the American pillar rose, that when shoots are cut into segments, there may be a gradient in rooting response from tip to base of one nature or another, depending upon the nature of the material. It is the purpose of this paper to show the gradient in chemical composition throughout the length of rose shoots used for propagation by cuttings. In addition, a comparison is made between plants grown with and without an abundant nitrogen supply.

Gradient in composition from tip to base

MATERIALS AND METHODS

One-year-old, field grown, *Rosa multiflora* (Thurnb.) plants propagated from one original plant by cuttings were placed in new, 12-inch clay pots in the greenhouse on January 23, 1931, all materials having been steam sterilized. The pots were set in shallow enameled pans, and the plants kept moist from below by maintaining a constant water level in the pans.

Six pots contained rich greenhouse soil high in nitrogen, as judged by plant growth. Six others contained quartz sand and were supplied with mineral nutrients once a week, but no nitrogen. By this means plants were grown for examination and analysis which were of contrasting appearance and composition—the one group with high nitrogen and the other with low nitrogen.

Both lots grew vigorously until the last of March, when the plants in quartz sand with no nitrogen began to slow down in growth, as evidenced by shorter internodes and yellowish foliage. The vigorous growth of these plants for 60 days, however, with no external supply of nitrogen, is interesting. The plants in rich soil were dark green at this time.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 16.

By May 4, 101 days after planting, the leaves of the plants receiving no nitrogen were yellowish green with reddish margins and the canes were stiff and yellowish green in color. By contrast, the leaves of plants receiving liberal supply of nitrogen were dark green, and the canes were deep green and flexible. At this time material was cut for chemical analysis.

Several canes approximately 100 cm. in length were taken from both the high-nitrogen and the low-nitrogen plants. The two lots were kept separate, and the canes were cut into sections 10 cm. in length and numbered consecutively from base to tip. The analysis of this material is given in table I. The material from the high-nitrogen or soil-grown plants

TABLE I

GRADIENT IN COMPOSITION OF ROSE SHOOTS FROM BASE TO TIP, GROWN WITH HIGH AND LOW NITROGEN

SAMPLE	DRY MATTER	FRESH MATERIAL		DRY MATERIAL	
		ASH	TOTAL NITROGEN	ASH	TOTAL NITROGEN
	%	%	%	%	%
PLANTS GROWN WITH LOW NITROGEN					
D1 (base)	58.38	0.992	0.180	1.70	0.31
D2	56.90	0.938	0.221	1.65	0.39
D3	56.78	0.919	0.181	1.62	0.32
D4	56.00	0.924	0.168	1.65	0.30
D5	56.95	1.013	0.193	1.78	0.34
D6	55.68	1.188	0.206	2.17	0.37
D7	49.09	1.055	0.181	2.15	0.37
D8	55.16	1.345	0.215	2.44	0.39
D9	54.87	1.525	0.235	2.78	0.43
D10 (tip)	55.34	1.887	0.298	3.41	0.54
PLANTS GROWN WITH HIGH NITROGEN					
L1 (base)	43.85	1.100	0.245	2.51	0.56
L2	41.00	1.070	0.278	2.61	0.68
L3	39.92	1.169	0.263	2.93	0.66
L4	39.91	1.217	0.275	3.05	0.69
L5	39.13	1.275	0.313	3.26	0.80
L6	36.90	1.335	0.302	3.62	0.82
L7	33.75	1.282	0.286	3.80	0.85
L8	32.64	1.361	0.300	4.17	0.92
L9	28.72	1.338	0.287	4.66	1.00
L10 (tip)	25.75	1.545	0.378	6.00	1.47

was numbered L1 to L10, from base to tip respectively. The material from the low-nitrogen or sand-grown plants was numbered D1 to D10, from base to tip respectively.

Leaves and petioles were removed and each lot cut into pieces 0.5 to 1 cm. in length, and placed in a covered aluminum moisture dish and dried to constant weight at 80° C., 25 mm. pressure. Total nitrogen and ash were determined by the Chemistry Department of this Station,[1] using the Kjeldahl-Arnold-Gunning method for total nitrogen.

Discussion

The data in table I need no detailed explanation. They show the general increasing gradient for moisture, ash, and total nitrogen from base to tip of rose shoots. They also show the higher moisture, ash, and total nitrogen content of the high-nitrogen plants over the low-nitrogen ones.

How much steeper the gradient becomes as it nears the tip is shown by the analyses of tips in table II, to be compared with the tip cuttings D10 and L10 in table I. Although D10 and L10 were tip cuttings, they were nevertheless 10 cm. in length, whereas samples 4 and 5 in table II were the distal 2 cm. of the shoot. It would be interesting to know the condition within still narrower limits at and near the growing point.

Figures 1–6 show the distribution and proportionate amount of starch in shoots of similar composition to those used for analyses. The relation between starch accumulation and total nitrogen content is inverse; that is, although nitrogen content is greatest nearest the tip of the shoot, the starch content is lowest.

In the comparison of shoots from plants grown in high and low-nitrogen media, the accumulation of starch is less in the high-nitrogen grown shoots.

Accumulation seems to occur first in the xylem parenchyma, next in the xylem rays, next in the perimedullary zone, next in the cortex parenchyma, next in the pith, and last abundantly in the cortex parenchyma. It is found sparingly in the phloem and rarely in the cambial zone, either fascicular or interfascicular.

In the sections showing greatest accumulation of starch, the greatest abundance is to be found in the xylem rays, perimedullary zone, and cortex parenchyma.

Chemical composition of rose shoots grown with high and low nitrogen

Materials and methods

The material used for analysis was grown and prepared for analysis as already described. The shoots from low-nitrogen plants growing in quartz sand were divided into two lots, nos. 1 and 2. The shoots from the high-nitrogen plants growing in soil were divided as nos. 3 and 6. Entire shoots were used in these analyses, with the exception of 2 cm. of succulent tips, which were cut from each lot and analyzed separately as nos. 4 and 5. The data are given in table II.

[1] We are indebted to F. J. Kokoski for these analyses.

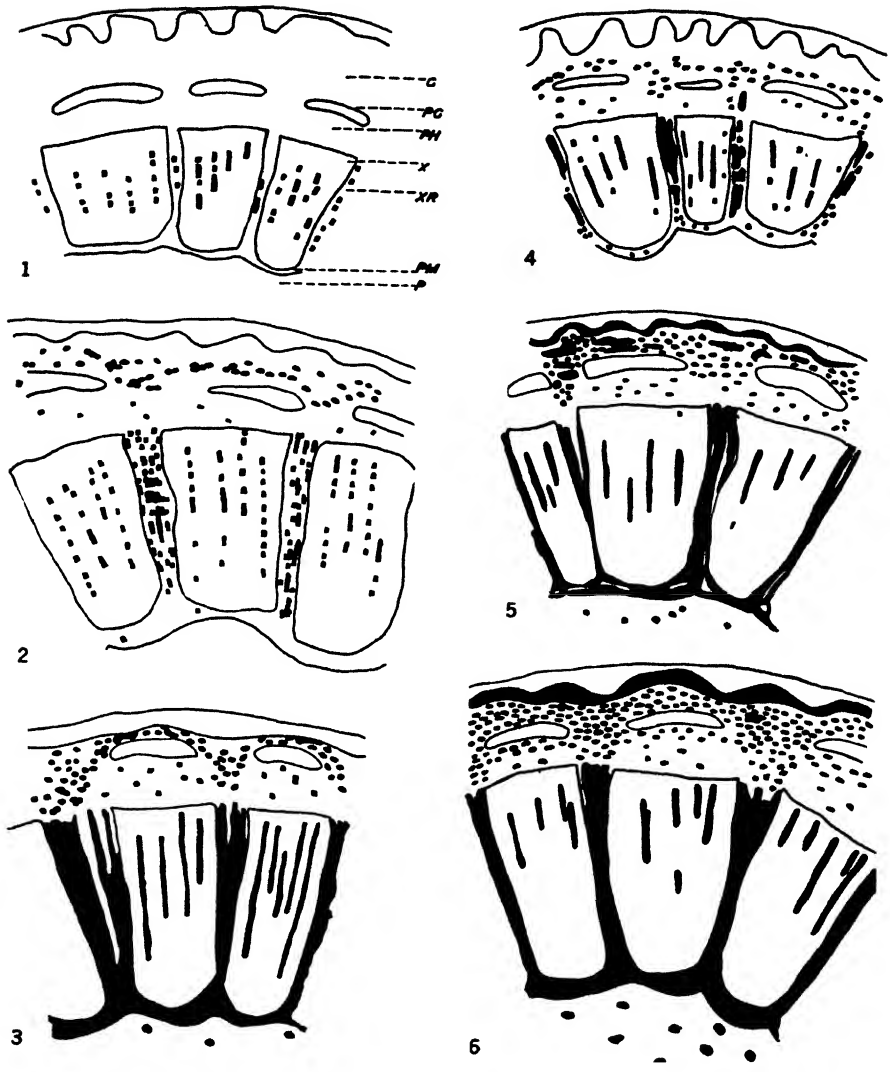
TABLE II

IE ANALYSIS OF ROSE SHOOTS GROWN IN HIGH AND LOW NITROGEN

Sample	Material	Dry matter	Fresh material						Dry material				
			Total sugar	Starch	Crude fiber	Unidentified alcohol-insoluble residue (hemicellulose)	Total N	Alcohol insoluble N† / Alcohol soluble N	Total sugar	Starch	Crude fiber	Unidentified alcohol-insoluble residue (hemicellulose)	Total N
		%	%	%	%	%	%	%	%	%	%	%	%
1	Grown in low N	49.36	1.367	4.59	12.80	26.44	0.177	6.6	2.77	10.09	25.93	52.79	0.3587
2	Grown in low N	50.39	1.445	6.23	19.40	19.03	0.2051	8.2	2.87	12.36	38.50	37.76	0.4070
3	Grown in high N	38.65	1.337	1.84	14.00	15.67	0.3812	4.9	3.46	4.76	36.22	40.55	0.9860
6	Grown in high N*	37.71	1.041	1.95	13.24	15.05	0.3144	6.9	2.76	5.17	35.11	39.91	0.8340
5	Tips from plants grown in low N	28.54					0.376						1.32
4	Tips from plants grown in high N	21.96					0.546						2.49
7	Tips from plants grown in high N*	23.02					0.464						2.01

* Two-year-old plants, intermediate between the other high- and low-nitrogen plants.

† The nitrogen in the hot 80 per cent. alcohol-insoluble residue is regarded as "protein" although the use of this term is not free from objection.



FIGS. 1–6. Sectors of transverse sections of rose shoots, showing starch accumulation: figs. 1–3, tip, middle, and basal sections respectively, from a high-nitrogen-grown plant; figs. 4–6, tip, middle, and basal sections respectively, from a low-nitrogen-grown plant.

Each lot was cut into pieces 0.5 to 1 cm. in length, and placed in a covered aluminum moisture dish and dried to constant weight at 80° C., 25 mm. pressure. The lots of succulent tips, namely, nos. 4 and 5, were then run for total nitrogen. The larger lots, nos. 1, 2, 3, and 4, were ground and again dried and weighed to determine the loss in grinding. Exactly 1 gm. of calcium carbonate was added and the whole sample extracted with hot 80 per cent. alcohol in a Soxhlet apparatus. Reducing sugars, sucrose, starch, and alcohol-soluble and alcohol-insoluble materials were determined by the methods used generally for plant materials at this Station (**2**), determining the reducing sugars by Bertrand's method (**1**) and the starch by taka-diastase followed by acid hydrolysis. Corrections were applied to allow for the loss in grinding and for the addition of the calcium carbonate. Crude fiber was determined by the official method.

Discussion

There is a high percentage of starch in the shoots from plants grown in the medium low in nitrogen (table II), and a lesser amount of starch in the shoots from plants grown in that high in nitrogen. The higher percentage of total nitrogen in shoots from plants grown in high-nitrogen soil is to be expected. Likewise the lower ratio of alcohol-insoluble nitrogen to alcohol-soluble nitrogen is to be expected, inasmuch as theoretically there should be a higher proportion of amino acids and other non-protein forms of nitrogen in actively growing parts.

These results emphasize the differences in composition between sections of a rose shoot 100 cm. in length. When shoots from plants of different growth habit are made into cuttings, there may be as much variation in composition between cuttings from the same shoot as between cuttings from different plants. Accordingly it would seem advisable to keep cuttings from a single shoot in numerical order from base to tip, and to compare the rooting habit of the sequence of cuttings as a whole rather than to make a percentage valuation as though it were a random sample.

Summary

1. Shoots of *Rosa multiflora* Thurnb., 100 cm. in length, when cut into 10-cm. sections, show a gradient of increasing moisture, ash, and total nitrogen content from base to tip, and a gradient of decreasing starch content.

2. Shoots from plants grown in a high-nitrogen medium show higher moisture, ash, and total nitrogen content, and lower starch content throughout their lengths than shoots from similar plants grown in a low-nitrogen medium.

3. The results of chemical determinations of rose shoots are presented, including dry matter, total sugar, starch, crude fiber, unidentified alcohol-insoluble residue, total nitrogen, and the ratio between alcohol-insoluble and alcohol-soluble nitrogen.

4. Sections of rose stem are reported with as much as 12.36 per cent. starch and only 0.4070 per cent. nitrogen on the dry basis; others with as much as 2.4 per cent. total nitrogen, and others with only 4.71 per cent. starch.

5. Starch accumulates first in the xylem parenchyma, next in the xylem rays, next in the perimedullary zone, next in the cortex parenchyma, next in the pith, and last in the cortex parenchyma abundantly. It is found sparingly in the phloem and rarely in the cambial zone. The greatest storage is to be found in the xylem rays, perimedullary zone, and cortex.

6. In view of the wide differences in composition, it would seem advisable in propagation experiments to keep cuttings from a single shoot in numerical order, and to compare the rooting habit of the sequence of cuttings as a whole rather than to make a percentage valuation as though it were a random sample.

STATE AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK
U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

LITERATURE CITED

1. KERTESZ, Z. I. Recalculated tables for the determination of reducing sugars by Bertrand's method. Privately published. Budapest. 1930.
2. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Association of Official Agricultural Chemists. Privately published. Washington, D. C. 1925.
3. SCHRADER, A. L. The relation of chemical composition to the regeneration of roots and tops on tomato cuttings. Proc. Amer. Soc. Hort. Sci. **21**: 187–194. 1924.
4. ZIMMERMAN, P. W. Vegetative plant propagation with special reference to cuttings. Proc. Amer. Soc. Hort. Sci. **22**: 223–228. 1925.

SIGNIFICANCE OF TRANSPIRATION[1]

HARRY F. CLEMENTS

Whether transpiration is beneficial or harmful to the development of plants is a much debated question. CURTIS (11) contends that it is almost entirely harmful with essentially no helpful contribution to the physiology of plants. MILLER (15) appears to agree with CURTIS when he states that "it must be admitted that its harmful effects outweigh any of the benefits that may be attributed to the process." MAXIMOV (14) seems to feel that transpiration is less helpful than it is harmful, suggesting that plants could adjust themselves to conditions which would prevail were transpiration non-existent. BARNES (2) in 1902 wrote that as plants developed on land they had to contend with this necessary evil. Others have reasoned that transpiration must be beneficial since all processes of life are of value. SHULL (18, 20) and others have shown the effect transpiration has on lowering temperatures.

I wish to present certain data which pertain to this subject and which may be divided into the following topics: transpiration as it reduces leaf temperatures; transpiration as it buffers leaf temperatures; transpiration as it enables rapid movement and distribution of inorganic solutes; transpiration as it aids photosynthesis; transpiration as it makes possible rapid, tall growth of plants; transpiration as a fact.

Transpiration as it reduces leaf temperatures

A tremendous amount of work has been done to determine the effect of transpiration on leaf temperatures. One procedure has been to determine the temperature of the leaf and compare it with that of the surrounding air. Thus CURTIS (11) points out that these obtained differences show that transpiration is of little significance since leaves usually have a higher temperature than the surrounding air. A moment's thought will show, however, that a comparison of leaf and air temperatures cannot be made since the absorption coefficient of the leaf is unlike that of the air. The capacity of the air to absorb light and radiate heat is very low, while the absorption capacity of plants is relatively high. MIX (16) reports that the sides of tree trunks exposed to the sun in winter with air temperatures below freezing will have temperatures as much as 39° F. above the shaded sides. HARVEY (12) observed that in winter a passing cloud will cause the tem-

[1] Contribution no. 36 from the Botany Department of the State College of Washington.

Presented before the Botanical Section of the Pacific Coast Branch of the American Association for the Advancement of Science. Pullman, Washington.

perature of exposed tree bark to drop as much as 18° F. within three minutes. Thus, although comparisons of leaf and air temperatures are interesting as ecological facts, they cannot in any sense be applied to the conclusion that transpiration does little or nothing to control leaf temperature.

Leaf temperatures have also been obtained for wilted and turgid leaves. CLUM (8) compared the temperatures of vaselined leaves, normal leaves, and dried leaves and found small differences. However, he made no effort to determine whether or not vaseline stops transpiration, nor did he determine whether or not the coefficient of absorption is the same in dry and turgid leaves. It is significant, however, that severely wilted leaves burn very quickly if not furnished water or shade.

Another type of procedure has been to calculate the amount of heat that would exist were transpiration not a fact. BROWN and ESCOMBE (5) measured the total radiant energy falling on unit area of leaf surface and from this determined the rise in temperature of a leaf, recognizing the coefficient of absorption, the mass of a leaf per unit area of surface, and the specific heat of the tissue. They failed to consider adequately the amount of light reflected from the leaf. This SHULL (19) has done. BROWN and ESCOMBE (5) therefore conclude that without some means of thermal dissipation, the temperature of the leaf would rise at the rate of 35° C. per minute. Obviously thermal emissivity would prevent this rise from continuing very far, but, as I shall show, lethal temperatures would soon be reached. SHULL (20) has shown the possible temperature increase to vary from a few degrees in thick leaved plants to many degrees in thin leaved plants.

One preliminary experiment reported by BLACKMAN and MATTHAEI (4), although set up for another purpose, appears to be the only experiment on record which demonstrates the influence of transpiration on leaf temperature in which living leaves were used. A leaf was placed inside a very small glass cage. The petiole of the leaf was dipped into water so that the leaf would not wilt. The whole container was closed so that the only way that heat could be lost would be by emission from the glass. When this set-up was placed in bright sunlight, the temperature of the leaf rose quickly to 52° C., at which time the experiment was discontinued because brown spots were appearing and the leaf was being killed. Careful examination of this experiment will show that, although the leaf was allowed to transpire, transpiration was really eliminated so far as temperature determinations were concerned. As the leaf absorbed radiant energy, partially reduced in intensity by the glass, the transformed energy in the form of heat vaporized water and kept the temperature of the leaf down. However, as soon as the atmosphere was saturated and as more vapor entered it, a super-saturated condition resulted, and as condensation of this vapor took place, the liberated heat offset the heat originally used in transpiration; and since transpi-

ration existed in equilibrium with condensation, the increase in temperature was the same as would have been the case had the leaf not been transpiring at all. It is significant that the leaf was killed by the increase in temperature.

It appears that transpiration plays a very prominent part in reducing the temperature of leaves to air temperatures. In succulents such as *Opuntia, Sempervirens,* etc., where transpiration activity is very low, and where almost all of the heat disposed of is done so by means of thermal emissivity, ASKENASY (1) reports temperatures of 43.7° to 51.2° C., approximately 20° C. above air temperature. PEARSON (17) found in extreme cases temperatures ranging from 10° to 20° C. above air temperatures in tissues of *Aloe* and *Euphorbia.* URSPRUNG (22) found that thin leaved plants in which transpiration is more active rarely exceed air temperatures by more than 5° C.

Transpiration as a temperature buffer

MAXIMOV (14) warns that if leaves were constantly heated to high temperatures, the plants would soon adjust themselves to this factor. There would, however, be a danger in such temperatures. Protoplasm is itself a delicately balanced colloidal system which cannot resist wide, sudden fluctuations in temperature. CLUM (9), in watching the continuous temperatures of leaves, reports the largest fluctuation in 15 minutes to be 5° C. In previous work (6) light intensities were determined by means of a black-bulb thermometer inserted in a vacuum. The readings were made in Centigrade degrees. Invariably at night the temperature obtained from this instrument was the same as that recorded on an ordinary thermometer, but during the day there was a difference of as much as 35° C. in the readings of the two instruments. The point to be emphasized here is that the smallest cloud passing across the sun's path would cause sudden and enormous changes in the readings of the black-bulb thermometer, in one case 26° in 10 minutes in the vacuum thermometer, whereas the air temperatures remained essentially uniform. The black-bulb thermometer varied as much as 30° C. in 30 minutes while the air temperature in the same period changed about 2°. Since leaf temperatures are known to follow air temperature closely, it is obvious that the transpiration intensity fluctuates according to the fluctuations in light intensity. This then is another important rôle of transpiration: it makes possible a uniform temperature of leaves and serves as a buffer which in its variation in rate absorbs the irregularities of sunlight. Thus, although MAXIMOV (14) believes that plants could adjust themselves to a higher temperature, it is extremely doubtful whether they could maintain a highly active metabolism and at the same time adjust themselves to an extremely variable temperature curve.

It may be pointedly remarked that no such plants appear to exist to-day. Plants such as *Opuntia* with high tissue temperatures maintain a uniform temperature curve because of their massiveness, but thin leaved plants with high internal temperatures have not been observed. This would seem to be significant.

Transpiration as it enables rapid movement and distribution of inorganic solutes from roots to leaves

CURTIS, in arguing that transpiration is a necessary evil to plants, disposes of the value of transpiration as a rapid distributor of inorganic solutes by stating that the salts move upward through phloem tissue and therefore not in the transpiration stream, which apparently moves through xylem tissue. In a previous paper (7) the logic in CURTIS's experiments with which he attempted to prove this point has been criticized, and data were presented which are briefly reviewed here. CURTIS's experiments were as follows: He selected twigs of plants in pairs and girdled one. The other twig was allowed to continue growth unharmed until the girdled twig was removed. Both twigs were then analyzed to determine the ash and nitrogen contents. His results invariably showed that the girdled twig has less of both materials in it than has the ungirdled twig. He concludes that the removal of a strip of bark around the twig, which breaks the continuity of the phloem, has interfered with the upward movement of the inorganic materials. This conclusion is correct, but he further concludes that this movement takes place in the phloem. This conclusion is logically unrelated to his first conclusion and also unrelated to his data, for he makes no effort to determine the amount of material which has moved through the girdle. In my experiments, one twig or cane of a pair was girdled and the other removed at the time of girdling. Analysis of these two twigs or canes demonstrated, first, the amount of material present above the girdle at the time of girdling, and second, the amount of material present above the girdle after the girdled twig or cane had grown through the experimental period. In every case a large increase in ash and nitrogen demonstrated that the movement of these materials is chiefly in the xylem. Without question a girdle interferes with the movement in the xylem, but only in some cases does it stop the movement and then only when it stops the water movement as well. Reasons for this interference appear to lie in the anatomical relations of the water conducting tubes.

Thus, contrary to CURTIS'S statement that the transpiration stream does not carry the salts, results reported elsewhere demonstrate that it does.

Transpiration as it affects speed of growth and stature of plants

Whatever the process may be by which soil solutes enter the living cells of plants, it is probably the same whether the plant is transpiring rapidly,

slowly, or not at all. What effect various rates of water loss may have on this process has never been satisfactorily determined. However, as soon as these solutes have entered the xylem they are in the transpiration stream and move upward with the water. SHULL (**10**) has pointed out that if molecules moved only by their own kinetic forces, the movement up trees would be extremely slow. He further points out, "It is the transpiration that gives rise to the mass movement of the water in the tracheal system, and in this current the ions and molecules are swept along." The advantage of transpiration here then is that it allows for a rapid distribution of mineral solutes once these have entered the plant. Furthermore, since this is true, a more rapid growth mechanism results in a greater extension of the root system with its resulting greater absorption.

MAXIMOV (**14**), in stating that plants would adjust themselves to higher temperatures if they existed, apparently forgot that in the environment of plants there are some unchanging constants. For example, were plants heated to 55°–60° C., would other factors remain in the same relation as at present? Probably not. BLACKMAN (**3**) and others have shown that when the temperature is favorable for photosynthesis, 25°–30° C., CO_2 absorption is the limiting factor. One can at once see the effect temperatures between 55° and 60° C. would have on the absorption of this important gas. The solubility of CO_2 at 15.6° C. in 100 volumes of water is 100.5 volumes; at 26.7° C., 68.6 volumes; and at 65.6° C., 11.4 volumes. Thus the absorption of the gas would be decreased about 84 per cent.; and since CO_2 absorption is already the limiting factor at 30° C., at 65° C. it would make rapid growth impossible. Furthermore, if thermal emissivity were the only means of thermal dissipation, the added 10°–20° C. of temperature would similarly retard photosynthesis. In this way the limitations of salt movement and CO_2 absorption without transpiration would give, instead of the luxuriant vegetation characteristic of regions where water is plentiful, a sparse, small, and slow growing flora characteristic of the desert.

Transpiration as a fact

Transpiration has unquestionably been a fact since plants originated. It is due to a property of water, namely, vapor tension, and as soon as terrestrial plants developed, they were influenced by it. It would indeed seem strange if today, after many years of evolution, the development of plants had not profited by one of the fundamental properties of water. Water plants do not contend with the same factors as land plants, of course, for although they may become very large, they are never farther removed from the source of water and salts than by the distance across a few cells. Land plants have not only been benefited by transpiration, as already pointed out, but they are dependent on it to keep them in proper nutritional balance after they have once become adapted to a given environment. Al-

though plants of a xeromorphic nature could utilize more water than is usually available in their native haunts, to which they are driven by the more efficient users of water, there is a limit to the amount of water any land plant can use. Plants characteristic of regions with 20 inches of rainfall and bright sunshine produce a certain yield of fruit, but when grown in regions of much rainfall and cloudy weather they develop a rank, succulent growth which fruits either not at all or only sparsely. Transpiration here serves as a controller of the internal nutritional status of the plant; for as KRAUS and KRAYBILL (13) have shown, the carbohydrate-nitrogen relationship can be markedly changed when water utilization is altered.

Even when transpiration must be reduced, it itself brings about profound changes within the plant to accomplish this end; for as SPOEHR (21) has shown, the pentose metabolism of succulents is a function of water deficit. Since succulence is of advantage to life on the desert, the process which provides for it must likewise be advantageous.

Further, plants characteristic of the temperate and arctic zones must withstand freezing temperatures through a part of the season. Plants with an over-supply of water late in the fall are invariably more susceptible to winter killing than plants growing under conditions favoring a limited supply or a liberal loss of water. Here, too, transpiration plays a part.

In the course of this discussion several beneficial functions of transpiration have been pointed out with respect to plants as we know them today: (1) It is a cooling agent. (2) It makes possible a buffered temperature curve. (3) It serves as a rapid distributor of soil solutes once they are within the xylem. (4) It enables the rapid growth of plants by (a) hastening salt distribution in the plant, and (b) cooling the leaves and thus aiding the solubility of CO_2 in plants and thereby increasing the rate of photosynthesis. (5) It serves as a stabilizer of the internal nutrition of plants with respect to both fruiting and tolerance of unfavorable conditions.

But there is no process in physiology which is not detrimental to the organism at some time. Osmosis when too active may cause splitting of various plant parts. Photosynthesis may be harmful to plants growing in infertile soils by causing accumulations of carbohydrates and ultimately bringing about the death of the plant. Respiration, too, may be harmful when too intensive. In a similar fashion, transpiration may cause the death of a plant which is so constructed as to use it. But such harmful effects are not so much due to the process itself as they are to the limiting factors which make the normal functioning of an essential process impossible.

STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

LITERATURE CITED

1. ASKENASY, E. Ueber die Temperatur, welche Pflanzen im Sonnenlicht annehmen. Bot. Zeitung **33**: 441–444. 1875.
2. BARNES, C. R. The significance of transpiration. Science n. s. **15**: 460. 1902.
3. BLACKMAN, F. F. Optima and limiting factors. Ann. Bot. **19**: 281–295. 1905.
4. ————, and MATTHAEI, G. L. C. Experimental researches in vegetable assimilation and respiration. IV. A quantitative study of carbon-dioxide assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. London. B **76**: 402–460. 1905.
5. BROWN, H. T., and ESCOMBE, F. Researches in some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings. Proc. Roy. Soc. London. B **76**: 29–111. 1905.
6. CLEMENTS, H. F. Hourly variations in carbohydrate content of leaves and petioles. Bot. Gaz. **89**: 241–272. 1930.
7. ————. The upward movement of inorganic solutes in plants. Res. Studies State Coll. Washington **2**: 91–106. 1930.
8. CLUM, H. H. The effect of transpiration and environmental factors on leaf temperatures. I. Transpiration. Amer. Jour. Bot. **13**: 194–216. 1926.
9. ————. The effect of transpiration and environmental factors on leaf temperatures. II. Light intensity and the relation of transpiration to the thermal death point. Amer. Jour. Bot. **13**: 217–231. 1926.
10. COULTER, J. M., BARNES, C. R., and COWLES, H. C. A textbook of botany. II. Physiology. Revised and enlarged by C. A. SHULL. Amer. Book Co. New York. 1930.
11. CURTIS, O. F. What is the significance of transpiration? Science n. s. **63**: 267–271. 1926.
12. HARVEY, R. B. Cambial temperatures of trees in winter and their relation to sun scald. Ecology **4**: 261–265. 1923.
13. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
14. MAXIMOV, N. A. The plant in relation to water. Edited with notes by R. H. YAPP. Allen and Unwin, Ltd. London. 1929.
15. MILLER, E. C. Plant physiology. First ed. McGraw-Hill Book Co. New York. 1931.

16. Mix, A. J. Sun scald of fruit trees: a type of winter injury. Cornell Agr. Exp. Sta. Bull. 392. 1916.
17. Pearson, H. H. W. Observations on the internal temperatures of *Euphorbia virosa* and *Aloe dichotoma*. Ann. Bolus. Herb. **1**: 41–66. 1914.
18. Shull, C. A. Transpiration as energy dispersal. School Sci. and Math. **19**: 1–6. 1919.
19. ————. A spectroscopic study of reflection of light from leaf surfaces. Bot. Gaz. **87**: 583–606. 1929.
20. ————. The mass factor in the energy relations of leaves. Plant Physiol. **5**: 279–282. 1930.
21. Spoehr, H. A. The carbohydrate economy of cacti. Carnegie Inst. Washington Pub. 287. 1919.
22. Ursprung, A. Die physikalischen Eigenshaften der Laubblätter. Bibl. Bot. **60**: 1–115. 1903.

EFFECT OF SOFT X-RAYS ON GERMINATION OF WHEAT SEEDS

HARRIS M. BENEDICT AND H. KERSTEN

(WITH SIX FIGURES)

Introduction

The effect of x-rays on the growth of seeds has been the subject of much investigation in recent years. Mutations, such as reported by GOODSPEED (3) in the case of tobacco, and changes in the rate of growth of the plants have been the principal results. While the literature has become too extensive to quote completely here, it should be noted that SHULL and MITCHELL (7), JOHNSON (4), and others state that small doses cause an increase in the rate of growth while LALLEMAND (6), CATTELL (2), and JOHNSON (5) claim that such doses cause no increase. All agree that large doses decrease the rate of growth.

These investigators used the ordinary Coolidge tube as a source of radiation, and thus made use of the hard, or short wave-length x-rays. The writers had available a gas x-ray tube having a window which transmitted the soft or long wave-lengths, and which could be operated at a voltage too low to produce the hard radiations. With this equipment available it was decided to irradiate wheat seeds with soft x-rays and carry out some of the determinations commonly made on seedlings.

Some preliminary experiments on the effect of these x-rays on the growth of wheat seeds, previously soaked in water, showed that when large doses were administered the wheat germinated until the coleoptile was about 2 cm. long, and remained this size for about ten days, after which it died. This might indicate, among other things, that the rayed seed had difficulty in utilizing the stored food; that is, that its starch was not so readily converted into sugar as in the unirradiated control seeds, and that respiration of the sugar was diminished. It might also indicate that the absorption of water necessary for germination was reduced.

To test these possibilities, the diastatic activity, reducing sugar content, respiration rate (as determined by comparing the dry weight before and after germination), and the percentage of water at the end of the germination period were determined for seeds irradiated for various lengths of time.

Methods

The x-ray equipment consisted of a copper target gas x-ray tube operated from a full-wave Kenetron-rectified alternating current supply at 10 milliamperes and 18 peak kilovolts. The window of the tube consisted of 0.035 mm. of aluminum and 0.04 mm. of cellophane, located about 3 cm.

from the focal spot. The seeds were held in special holders with their embryo ends toward the target of the tube and 1 cm. distant from the window. This arrangement permitted a lead shutter to operate between the seeds and window as shown in figure 1. This window easily transmitted

FIG. 1. X-ray tube window, shutter, and seeds in the holder.

the characteristic K_α and K_β (1.537 Å and 1.389 Å) lines of copper. Figure 2 shows a photograph of the radiation made in an oscillating rock-

K_β K_α

FIG. 2. Spectrogram of the radiation used, showing the K_β and the K_α lines of the copper target.

salt crystal spectrograph in which the two lines stand out strongly, indicating that the radiation was nearly monochromatic.

The wheat seeds (var. Purdue 352) were soaked for 12 hours in distilled water, at the end of which time they were found to contain an average of 28 per cent. water. They were then irradiated, sterilized in 10 per cent. formalin for 20 seconds, and placed between blotters in zinc trays to germinate in the dark for seven days. The trays had previously been sterilized with 0.1 per cent. mercuric chloride solution. After germina-

tion the seeds were dried for 48 hours at 40° C. and the following determinations made.

The diastatic power was determined by the Wohlgemuth iodine method. The dried seedlings were ground to a powder and extracted with distilled water (using 15 cc. per gram of powder) for three hours, with occasional stirring. Various quantities of the filtered liquid extract were added to 5 cc. of soluble starch solution and the enzyme allowed to act for three hours at 40° C.

The total reducing sugar was found by determining the amount in the enzyme extract and the amount left in the residue and adding them together. The sugar in the residue was extracted with alcohol according to the method described in the A.O.A.C. (1). The copper was reduced by the Munson-Walker method and determined by the volumetric permanganate method. The amount of material respired was obtained by subtracting the dry weight of the germinated seeds from the original dry weight of the seeds.

Each determination was made on 200 seeds.

Results

In figures 3–6, the results of the various determinations are plotted as functions of the time of irradiation of the seeds. The heavy vertical lines represent the limits of variation of the quantity plotted; the large circles represent the arithmetical averages of the corresponding sets of values; the numbers in the large circles represent the numbers of determinations made. For example, in figure 3, corresponding to 15 seconds, it is seen that six determinations were made. Since each determination was made on 200 seeds, there were 1200 seeds used. The values obtained vary from 15 to 25, but the arithmetical average, as shown by the position of the large circle, is 22. The small circles represent determinations when only a single value was obtained.

The results of the diastase determinations are shown in figure 3. The diastatic activity, determined by the number of grams of soluble starch decomposed per gram of dry seedlings per hour at 40° C., is plotted against the time of irradiation in seconds. In figure 4 are plotted the results of the reducing sugar content of the seedlings. The ordinates represent the milligrams of sugar present per gram of dry weight of seedlings. The respiratory rates of the seedlings are shown in figure 5. The milligrams respired per gram of original dry seed is plotted against the time of irradiation. Figure 6 shows the percentage of water in the seedlings at the end of the germination period.

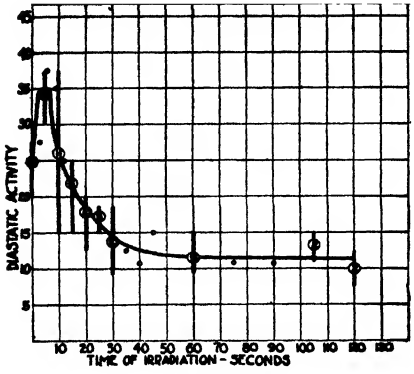


FIG. 3

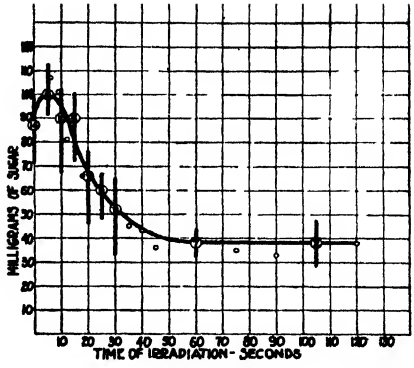


FIG. 4

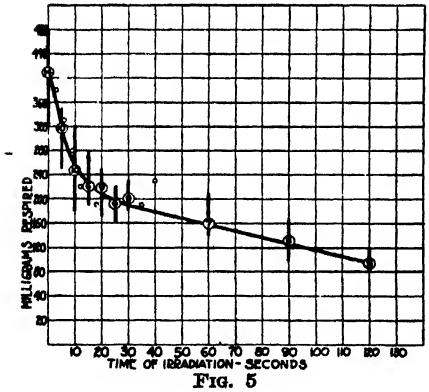


FIG. 5

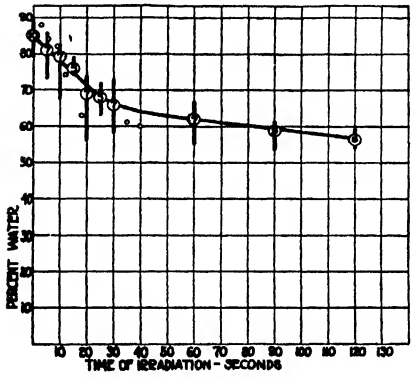


FIG. 6

FIG. 3. Diastatic activity (number of grams of soluble starch decomposed per gram of dry seedlings per hour at 40° C.) plotted against time of irradiation in seconds.

FIG. 4. Milligrams of sugar present per gram of dry weight of seedlings as a function of the time of irradiation.

FIG. 5. Milligrams respired per gram of original dry weight as a function of the time of irradiation.

FIG. 6. Percentage of water in the seedlings at the end of germination period as a function of the time of irradiation.

Discussion

The results of the diastase determinations show that there is an increase in the diastatic power during the five seconds of irradiation. That this is a true increase is shown by the fact that all the points on this line are above the highest point of the control. This does not necessarily mean that the activity of the diastase present is increased. It may mean that the ability of the seeds to produce diastase is changed by the irradiation, for the dosages of x-rays used had no effect on either wet or dry commercial taka-diastase.

The results of the sugar determinations are strikingly parallel to those of the diastase determinations. Since the function of the diastase is to convert starch to sugar, the curves for starch and sugar should be parallel and act as a check on each other.

The respiration rates, as determined by the loss of weight of seedlings while germinating, showed that the amount respired decreased with increased time of irradiation.

These three results, taken together, seem to indicate that seedlings grown from x-rayed seeds are increasingly hindered in the utilization of their stored food by prolonged irradiation.

The results of determinations of the water content of the seedlings indicate that the ability of the seeds to take in water decreases with increased time of irradiation.

Summary

1. Wheat seeds were irradiated with soft x-rays for different lengths of time, and the diastatic activity, the reducing sugar content, the respiratory rate, and the percentage of water in the seedlings were determined.

2. Wheat seedlings irradiated for five seconds show an increase both in diastatic activity and in sugar content, but if irradiated for longer time there is a decided and progressive decrease in these two substances.

3. The amount of material respired shows no increase over the controls and decreases as the time of irradiation increases. These results indicate that, under the conditions of this experiment, irradiated seeds cannot change their stored starch into sugar as readily as the controls except for very short periods of irradiation, and that even then the seeds cannot use the sugar for growth as readily as can the control seeds.

4. The percentage of water in the seedlings at the end of the germination period indicates that the irradiated seeds cannot take in water as readily as can the controls.

UNIVERSITY OF CINCINNATI
CINCINNATI, OHIO

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and Tentative Methods of Analyses. 3rd ed. Washington. 1930.
2. Cattell, W. The effect of x-rays on the growth of wheat seedlings. Science n. s. **73**: 531–533. 1931.
3. Goodspeed, T. H. The effect of x-rays and radium on species of genus *Nicotiana*. Jour. Hered. **20**: 243–249. 1929.
4. Johnson, Edna L. Effect of x-rays upon growth, development, and oxidizing enzymes of *Helianthus annuus*. Bot. Gaz. **82**: 373–402. 1926.
5. ————————. On the alleged stimulating action of x-rays upon plants. Amer. Jour. Bot. **18**: 603–614. 1931.
6. Lallemand, S. Étude de l'action des rayons X sur le développement des plantes. Arch. Anat. Hist. Embryol. **10**: 1–233. 1929.
7. Shull, C. A., and Mitchell, J. W. Stimulative effects of x-rays on plant growth. Plant Physiol. **8**: 287–296. 1933.

BRIEF PAPERS

ABSORPTION SPECTRA OF ALPHA AND BETA CAROTENES AND LEAF XANTHOPHYLL AT ROOM AND LIQUID NITROGEN TEMPERATURES [1]

A method was developed whereby the absorption spectra of photosynthetic pigments in the solid state (glasses) were photographed with a Steinheil spectrograph. The apparatus employed was a quartz Dewar tube furnished with four plane quartz windows. The carotenoid solutions were placed in 1 mm. thick pyrex cells, which, after pre-cooling in ether at – 116° C., always were immersed in the refrigerating liquid. The cells were fastened to a metallic tube which could be raised and lowered until the cell was in the proper position. The source of continuous radiation was a 500-watt Mazda lamp operating on a 110-volt direct current. The respective sample was first photographed at room temperature, then at liquid nitrogen temperature. The carotenes were dissolved in ligroin (b. pt. 30°–35°) and the leaf xanthophyll in anhydrous diethyl ether. The error in reading band limits was between 4 and 5 per cent. The results are summarized in table I.

TABLE I

ABSORPTION BANDS OF ALPHA AND BETA CAROTENES AND LEAF XANTHOPHYLL AT LIQUID NITROGEN AND ROOM TEMPERATURES

CAROTENOID	BAND LIMITS AT LIQUID NITROGEN TEMPERATURE	BAND LIMITS AT ROOM TEMPERATURE	DECREASE IN BAND WIDTHS AT THE LOWER TEMPERATURE	MAXIMA AT LIQUID NITROGEN TEMPERATURE	MAXIMA AT ROOM TEMPERATURE	SHIFT IN MAXIMA CAUSED BY LOWERING THE TEMPERATURE
	Å	Å	Å	Å	Å	Å
Alpha carotene	5050–4900	4890–4700	40	4975	4795	180
	4700–4600	4525–4380	45	4650	4452	198
	4440–4300	.....	.....	4570	.....	.....
Beta carotene	5050–4880	4870–4665	55	4955	4767	188
	4700–4560	4535–4545	50	4630	4440	190
	4404–4260	.....	.....	4332	.....	.....
Leaf xanthophyll	4980–4750	4850–4550	70	4865	4700	165
	4660–4460	4540–4270	70	4547	4380	167
	4345–4235	.....	.....	4290	.....	.....

The above data show (a) that absorption bands at liquid nitrogen temperature are appreciably decreased in width, (b) that bands which are difficult to recognize at room temperature become very distinct at the lower temperatures, and (c) that the maxima for carotenoids are shifted 165 to 200 Å toward the infra-red. This investigation is being extended to other photosynthetic pigments.—ELMER S. MILLER,[2] *University of Chicago.*

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] National Research Fellow in the Biological Sciences.

SOLUBLE SOLIDS IN THE WATERMELON[1]

(WITH ONE FIGURE)

In October, 1931, tests were made of the variations in percentage of soluble solids in different areas of a watermelon grown in the Snake River Valley near Payette, Idaho. This melon was a black-seeded strain of the Angeleno variety. It was cut in half and then in quarters, as shown in figure 1. Juice was squeezed from small pieces of pulp removed from each

FIG. 1. Distribution of sugars in the black-seeded Angeleno watermelon.

zone and tested for soluble solids by use of the Zeiss refractometer. Most of the soluble solids in a watermelon are sugars.

As is shown in the figure, the juice in the green area just under the

[1] Contribution from the Department of Horticulture, University of Idaho, no. 107.

rind was low in soluble solids, containing 4.1 to 6.8 per cent. The red area between this green portion and the whorls holding the seeds was higher, containing about 9 or 10 per cent. in this melon. The core area ranged next with about 11 to 12 per cent., and the area around the seeds highest, with about 11 to 15 per cent. The concentration seemed higher in the tissues leading to the seeds as compared with those leading elsewhere, and also higher near the large seeds than near the small seeds toward the blossom end.—LOWELL R. TUCKER, *Department of Horticulture, University of Idaho, Moscow.*

NOTES

The Boston Meeting.—The tenth annual meeting of the American Society of Plant Physiologists at Boston, December 28–30, 1933, was a notable meeting, and reflected the ever increasing interest in the physiology of plants. In spite of the severe weather, the attendance was good, and the papers presented were in many instances of more than average merit. Some of them were of unusual value and significance. The various sessions listed 51 papers originating in 34 institutions from all parts of the United States. As growth continues, it will probably be found advisable to organize parallel programs. If rooms in close proximity were assigned for such simultaneous programs, it would be relatively easy for one to transfer from one program to another at will. The advantages would be that a stricter classification of papers could be adopted, with fewer papers on each program, and with more time for deliberate discussion and exchange of ideas. The occasional apparent lack of interest in papers is usually produced by an overloaded program, which causes even interested listeners to hesitate to take time for critical suggestions, or to present alternative views. A program which allowed about as much time for discussion as for presentation of material would obviate these difficulties, and make the programs very much more worth while to the one whose work is presented. Occasionally some one travels a thousand miles to get the unbiased criticism of the entire group, and obtains not a single suggestion because the time is too brief, the number of papers too great, for the deliberate consideration of each. This is a matter for regret, and if parallel programs would relieve the condition, it would be a very advantageous change.

Life Membership Award.—The ninth award of the CHARLES REID BARNES life membership was made at the annual dinner of the Society to Dr. JAMES BERTRAM OVERTON, for many years professor of plant physiology at the University of Wisconsin. Dr. OVERTON was born at Richmond, Michigan, December 23, 1869. His first degree, Ph.B., was obtained at the University of Michigan in 1894. Some years later, 1900–1901, he was holder of a fellowship and assisted in botany at the University of Chicago, from which institution he received the Ph.D. degree in 1901. During this period he enjoyed intimate association with Dr. COULTER, Dr. BARNES, and other members of the staff at Chicago. Following his connection at Chicago, he was a research assistant with the Carnegie Institution, and Professor of Biology at Illinois College, Jacksonville, Illinois. In 1904, he went to the University of Wisconsin as instructor in botany. Here he has continued his service, as assistant professor of botany in 1907, associate professor of

plant physiology in 1912, and professor of plant physiology since 1915. At times he has resumed his connection with the Carnegie Institution as research associate. His early research was concerned with parthenogenesis, and he has maintained his interest in cellular behavior during fertilization and reduction division. His main contributions in physiology have concerned the movements of liquids and gases in plants. His early publications on this subject dealt with sap rise in *Cyperus*. During recent years he has shared in important contributions from the Carnegie Institution on sap flow and pressure in trees, distribution of gases in trees, physiological anatomy of stems, and methods of determining the pathway of transfer of solutes and sap, especially in woody species.

Amendment of the Constitution.—A slight change was made in the constitution of the Society to permit the organization of regional sections where such action seems desirable. Sectional organizations have proved to be a great stimulus to interest in physiological research wherever they exist. With a rapidly advancing science there is great need of free exchange of ideas and mutually helpful criticism. Sectional organizations are conducive to cooperative action, and their influence on the character of the research undertaken justifies their development wherever there is sufficient interest to maintain them.

New England Section.—A significant step was taken in granting the petition of New England members of the Society for the organization of a New England Section. A large group of members representing all of the New England states signed the petition. Following the granting of the petition, the New England members held an organization meeting to elect officers and plan their future work. Dr. CARL G. DEUBER, of Yale University, is first chairman of the new section, Dr. GEORGE P. BURNS, of the University of Vermont, is vice-chairman, and LINUS H. JONES, of the Massachusetts State Agricultural College, is secretary-treasurer. Plans are already being laid for a meeting of the New England section sometime late in May, 1934. The successful development of this activity in the field of plant physiology will doubtless be of great advantage to the botanical interests of all the New England states. The future growth of the new section will be watched with great interest.

Regulations of Publication.—Important new regulations regarding publication by the Society were adopted at Boston. The regulations have received unanimous approval by the Editorial Committee, by the Executive Committee, and by the Society as a whole at its business meeting. The regulations are published here to guide authors in submitting papers for

consideration. There are three essential limitations imposed for the good of all, as follows:

(a) Free space for any one contribution is limited to 20 pages. If papers exceed this limit, authors or their institutions must find financial support for the extra space. Papers of any length may still be used if of sufficient merit, but space in excess of 20 pages must be provided for in some way by the author.

(b) Space devoted to tables and cuts is limited to 5 pages in any single contribution. Additional cuts and tables may be used, but authors or institutions must guarantee payment for any excess above a 5-page limit for a single paper.

(c) Non-members are required to pay a fee of $10.00 for each paper published in PLANT PHYSIOLOGY. In explanation of this requirement, it should be stated that PLANT PHYSIOLOGY has been open to non-members on equal terms with members during the last 8 years. In one year 27 non-member authors were accommodated. In case of a choice the editors prefer good papers, regardless of where they originate, and must continue to use only the best material available. But good papers by members have to wait unduly long for publication as a result of this liberal policy. Members are contributing toward the costs of publication, while non-members have not done so. It is merely to equalize this responsibility toward the costs of publishing that non-members are now to be required to pay a fee for the privilege of using space.

In practice this rule is to be applied as follows: Only one fee will be required when there are two or more non-member joint authors to a paper. And in case the non-member is joint author with a member, no fee will be required. In this way students will not be penalized for becoming productive before they are financially independent. The regulations under (a) and (b) apply to non-members as well as members.

These regulations are much more liberal than some that have been adopted by other societies. The Society desires to maintain PLANT PHYSIOLOGY as a most liberal journal. If conditions change so that the restrictions are unnecessary at some future time, they may be considered as a temporary expedient.

Sixth International Botanical Congress.—The following notice has been received from the Secretary of the Congress:

"The Organization Committee of the VI International Botanical Congress from various sides has been asked to change the dates of this Congress; the Committee has now decided that the Congress will meet at Amsterdam, Holland, September 2nd–7th, 1935. A first notice regarding this

Congress has been sent out to a number of addresses; for additional copies please apply to the Secretary, Dr. M. J. SIRKS, Wageningen, Holland."

Plant Constitution.—Attention is called to a publication from the press of Paul Parey, Hedemannstrasse 28–29, S.W.11, Berlin, Germany. It is entitled Pflanzliche Konstitutionslehre, by F. MERKENSCHLAGER and M. KLINKOWSKI. It deals with the internal capacities of plants to respond to conditions of their environment. It also considers the geographic origin of species, and its influence on plant constitution, particularly on the physiological direction of development. From humid regions have come species of low metabolic intensity, while from arid origins have come species with a constitutionally greater activity and productiveness.

Among the plants of humid origin described in the work are the potato, serradella, oats, buckwheat, yellow lupin, and rye. Those of more arid origin include the white mustard, sugar beet, wheat, alfalfa, and barley. The geographic migrations of these domesticated species are depicted, and an attempt made to account for the present constitution of these forms. The book is quoted by the publisher in brochure only, at RM 7.50 per copy.

British Economic Grasses.—A very useful monograph on British grasses has been prepared by SIDNEY BURR and DOROTHY M. TURNER, of the University of Leeds. It carries a foreword by STAPLEDON. About 35 varieties and species are included in the monograph. It is a taxonomic work, but should prove extremely useful to agronomists and physiologists who work with these or any other species of grass. A brief introduction gives information on vegetative and anatomical characters of grasses in general. This is followed by two keys, one based on vegetative characters, the other on anatomical characters. Following the keys are brief descriptions of the species included, with cross sections of the shoots with leaves inrolled, and of expanded leaves. Those interested in grass research will find it a very helpful guide to the structural features in this family. More books of this type are needed, for both wild and cultivated species in other families. The work is published by Edward Arnold and Co., but is distributed in the United States by Longmans Green and Co., New York, at a price of $3.75 per copy.

Vol. 9 No. 2

PLANT PHYSIOLOGY

APRIL, 1934

NUTRITIONAL REQUIREMENTS OF THE ROOT-ROT FUNGUS, *PHYMATOTRICHUM OMNIVORUM*[1]

WALTER N. EZEKIEL, J. J. TAUBENHAUS, AND J. F. FUDGE

(WITH SIX FIGURES)

Introduction

A part of the general program of study of the destructive root-rot disease caused by *Phymatotrichum omnivorum* (Shear) Duggar has been a consideration of the nutritive relations of the fungus. The phenomenally wide range of host plants on which the root-rot fungus is an aggressive parasite suggests that knowledge of its nutritive requirements may add to our general concept of the parasitism of fungi, as well as increase our fundamental knowledge about this particular organism.

Phymatotrichum omnivorum has been grown in artificial culture at various times since ATKINSON (1) first isolated the fungus in 1892. He grew the fungus on sterilized sweet potato, cotton roots, apple roots, and manure. Later DUGGAR (3), TAUBENHAUS and KILLOUGH (17), and KING and LOOMIS (8, 9) described growth of the fungus on a wide variety of sterilized roots, stems, seeds, and fruits, and on agars prepared from such natural materials. The present work has been carried on concurrently with studies on the growth of the fungus in plant juices (6), and phases of the present work have been summarized previously (5).

Methods

The general plan has been to compare the growth of a single strain of *Phymatotrichum omnivorum* in flask cultures on the various synthetic media. Series of cultures were inoculated at the same time and incubated side by side at the specified temperatures. The dry weight of fungus

[1] Published with the approval of the Director as Contribution no. 215, Technical Series, of the Texas Agricultural Experiment Station.

growth produced was determined for each culture in which there was perceptible growth by filtering out the nutrient solution and drying the fungus colony in a crucible in the oven.

The strain of the fungus used in all the experiments was our no. 24, isolated August 6, 1929, from a sclerotium from a field at the Temple, Texas, Substation. This strain was proved parasitic and capable of causing typical root rot of cotton plants by successful inoculation experiments after 2 months and again after 27 months in artificial culture. Strain 24 has been carried in culture now for more than 2 years and 9 months and has shown no evidence of attenuation.

The first experiment was started from a tube culture of the fungus; but later series were inoculated with small pieces of sclerotia produced on the synthetic culture media. Sclerotial masses taken for this purpose were those produced on the bare walls of the flasks, above the level of the substratum, presumably precluding possibility of carrying portions of the old substrata over to the new cultures; and cultures for an entire experiment were always seeded with bits of sclerotia cut from the mass produced in a single flask. In practice, small portions of the sclerotial mass were pulled with a needle from the side of a flask, transferred to a petri dish containing moist, sterile, filter paper and cut with a scalpel into small pieces which were transferred to the cultures to be inoculated.

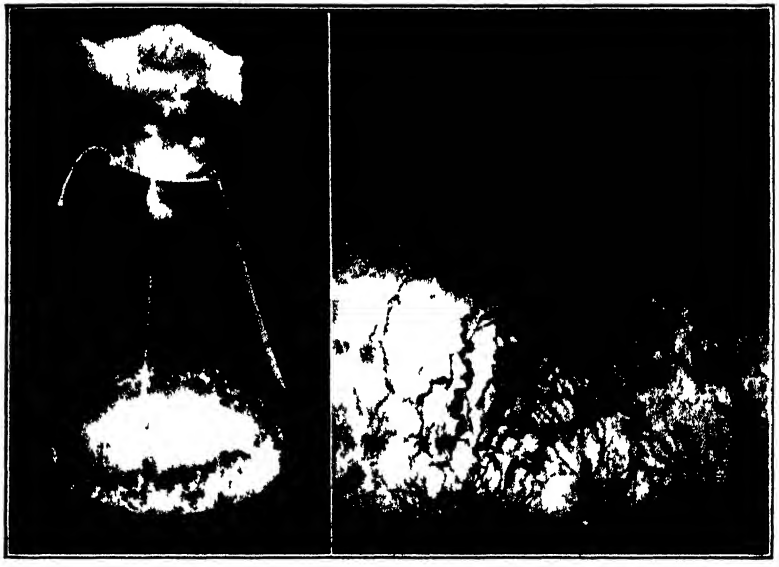
Chemicals used were of the chemically pure grade of Baker analyzed, Kahlbaum, or Difco products, but were not specially purified for this work. Unless otherwise specified, 50 cc. of the solution were used in each 250-cc. Erlenmeyer flask. Certain series included liquid nutrient solutions only; others included also parallel sets of agar slant or flask cultures, made up with 2 per cent. agar added to the respective solutions. The cultures were incubated on shelves in a large culture room, in which the temperature was held near 26°–28° C. during the winter by manipulation of the heating and ventilation, while in summer it rose to an average of 31°–33° C. The results within a given series are thus directly comparable but this is not true for results in different series.

The oven-dry weights of fungus growth were determined after the specified time by filtering the entire cultures through asbestos mats in Gooch crucibles in earlier work, and through alundum crucibles in the later series, washing rapidly with distilled water, and then drying in the oven at 90°–95° C. for 18 hours. The weights of colonies produced in replicate cultures were usually not very different, and only the average values have been tabulated. The reliability of these mean values is discussed in a later section.

Experiments

A preliminary experiment was started October 18, 1929, to find a liquid nutrient solution suitable as a starting point for the studies. Growth of

Phymatotrichum omnivorum was rapid and profuse in the artificial potato-dextrose medium devised by BROWN (**2**);[2] next in potato-dextrose decoction and agar; somewhat less in BROWN's simplified formula;[3] and none in SHIVE's "best" solution. Sclerotia developed profusely in anastomosing chains and matlike aggregations on the sides of the flasks in the complete BROWN's medium. This was the first record of production of sclerotia in pure cultures on synthetic media (fig. 1), although sclerotia had



A B

FIG. 1. *P. omnivorum* on synthetic media, showing masses of sclerotia: *A*, well developed sclerotial masses encircling flask culture 5 weeks old; *B*, sclerotia developing in chains and masses on wall of a 24 day-old culture.

been discovered some months earlier by KING and LOOMIS (**9**) in cultures on cotton and soil materials, and later found by NEAL (**12**) in the field. These sclerotia were used to inoculate succeeding series of flask cultures.

Later series were set up with various modifications of the complete BROWN's formula. In one series cultures were made with media which differed only by the omission respectively of one of the eight components of the complete formula. The fungus grew well in all the media except those from which starch or potassium phosphate had been omitted. In these two

[2] BROWN's complete medium (our solution no. 3) was made up to contain per liter (grams):—peptone 1.8, asparagin 1.8, dextrose 2.0, corn starch 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, KCl 0.15, $FeCl_3$ trace.

[3] BROWN's simplified medium (our solution no. 4) was made up to contain per liter (grams):—asparagin 2, dextrose 2, $MgSO_4$ $7H_2O$ 0.75, K_2PO_4 1.25.

media the early hyphal strands soon darkened and growth ceased, with no production of sclerotia; while with most of the other seven media, growth continued for several weeks and sclerotia appeared in many of the flasks.

Another series was used to compare growth of the fungus on media with constant nitrogen and mineral content but varying carbohydrate content. One solution included starch and a small amount of dextrose, as specified in the original BROWN formula; the other media included dextrose in amounts ranging from 2 to 40 gm. per liter. With the latter it was possible to filter off the substratum and to determine the weights of the colonies. Dextrose at 40 gm. per liter had supported the heaviest growth, averaging 106 mg. per flask after 16 days; while growth was less with lesser amounts of dextrose, and almost completely inhibited with only 2 gm. per liter or with none. Differences in the extent of growth corresponding to the differences in carbon content were observed also on agar slants made up from these nutrient solutions with the addition of agar (fig. 2). The growth

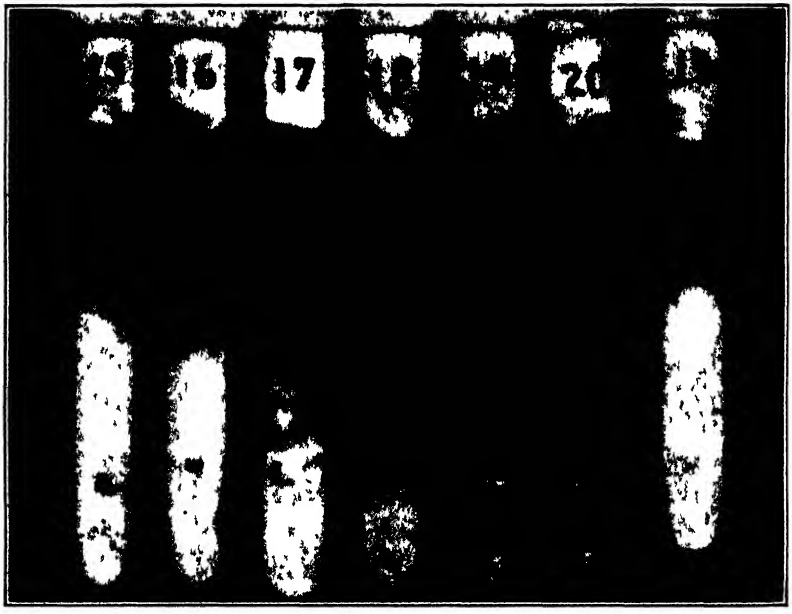


FIG. 2. Growth of *P. omnivorum* in 16-day-old cultures, on media with varying concentrations of dextrose (dextrose content decreasing from 40 gm. per liter in medium 15 to 20 gm., 10 gm., 5 gm., 2 gm., and finally to none in medium 20. Medium 14 contained 2 gm. of dextrose plus 40 gm. of starch).

obtained with dextrose was approximately as heavy as with the same concentrations of starch, and dextrose was adopted as the source of carbon for most of the later work.

MINERAL NUTRIENTS

The effects of variation in the mineral content of solutions on the growth of the fungus are summarized in table I. The sources of nitrogen (peptone and asparagin) and of carbon (dextrose) were constant, while the mineral ingredients were varied in a number of combinations. The completed media were adjusted originally to approximately pH 7.0, using KOH or HCl respectively, except that H_2SO_4 was used with solution 54, NaOH with solution 57, and lime water with solution 58. The first four formulas differed by the cumulative addition to the basic formula of (1) magnesium sulphate, (2) potassium chloride, and (3) potassium phosphate. There was marked increase in growth only in solution 49, which included all three salts in what may be termed the "standard" concentration. Solutions 50, 51, and 52 included these three salts, with one of them at ten times the standard concentration. This increase in concentration encouraged slightly increased growth with magnesium sulphate, and somewhat reduced growth with potassium chloride and with potassium phosphate. In solution 53 the sulphate concentration was maintained at the same level as in solution 49, but magnesium was omitted and potassium increased, and markedly lighter growth resulted. This occurred also with the omission of the phosphate ion (solution 56), with substitution of sodium for potassium and magnesium (solution 57), and with substitution of calcium for potassium and magnesium (solution 58). It should be noted with regard to solution 58, however, that precipitation of tricalcium phosphate during neutralization of this medium had probably affected the concentration of phosphates available to the fungus. No significant change in growth resulted from omission of chlorine in solution 54, and but a small reduction in growth occurred in solution 55, in which the sulphate ion was omitted and the chlorine increased. Throughout the series, omission of potassium phosphate reduced growth more than omission of any other mineral constituent (fig. 3). Significant, though smaller, decreases resulted from omission of magnesium alone or of magnesium plus potassium.

The series summarized in table II included combinations of potassium and magnesium phosphates, sulphates, and chlorides, as well as a few combinations of sodium and calcium salts, as additions to a solution of ammonium nitrate and dextrose. Before autoclaving, media for this series were mostly adjusted to approximately pH 5.5 (instead of pH 7.0 as in most series), to allow for greater solubility of the magnesium salts; however, magnesium phosphate precipitates formed in solutions 97, 98, and 99. No adjustment was necessary for solutions 93, 97, 98, 100, and 101; sodium hydroxide was used for solution 103; ammonium hydroxide was used for solution 102; and phosphoric acid was used for the remaining solutions.

TABLE I

PRELIMINARY COMPARISON OF MINERAL NUTRIENTS AS AFFECTING GROWTH OF THE FUNGUS (INCUBATED AT 31°–35°, MEAN = 33.1° C.)

Nutrient solutions			Growth, solid and liquid cultures		Final results in 6 flasks of liquid after 5 weeks	
No.	Materials added to basic formula,* gm. per liter		Mycelium	Sclerotia	Final reaction of media	Mean dry weight of colonies
		gm.			*pH*	*mg.*
46	0 (basic formula only)		++	–	6.4	72
47	$MgSO_4 \cdot 7H_2O$	0.75	++	–	6.4	67
48	$MgSO_4 \cdot 7H_2O$	0.75	++	–	6.4	73
	KCl	0.15				
49	$MgSO_4 \cdot 7H_2O$	0.75	++++	++	5.2	463
	KCl	0.15				
	K_2PO_4	1.35				
50	$MgSO_4 \cdot 7H_2O$	7.5	++++	++	4.8	523
	KCl	0.15				
	K_2PO_4	1.35				
51	$MgSO_4 \cdot 7H_2O$	0.75	++++	+	5.5	445
	KCl	1.5				
	K_2PO_4	1.35				
52	$MgSO_4 \cdot 7H_2O$	0.75	+++	–	6.0	414
	KCl	0.15				
	K_2PO_4	13.5				
53	K_2SO_4	0.52	++++	Trace	5.3	170
	KCl	0.15				
	K_2PO_4	1.35				
54	$MgSO_4 \cdot 7H_2O$	0.75	++++	Trace	5.5	485
	K_2SO_4	0.175				
	K_2PO_4	1.35				
55	$MgCl_2 \cdot 6H_2O$	0.62	++++	+	6.3	408
	KCl	0.15				
	K_2PO_4	1.35				
56	$MgSO_4 \cdot 7H_2O$	0.75	+	–	6.1	77
	KCl	1.57				
57	Na_2SO_4	0.43	++++	+	5.3	101
	NaCl	0.117				
	$NaH_2PO_4 \cdot H_2O$	0.88				
58	$CaSO_4$	0.405	++	–	6.3	81
	CaCl	0.111				
	$Ca(H_2PO_4)_2 \cdot H_2O$	0.82				

* Basic formula 46 contains per liter (grams): peptone 1.8, asparagin 1.8, dextrose 40.0.

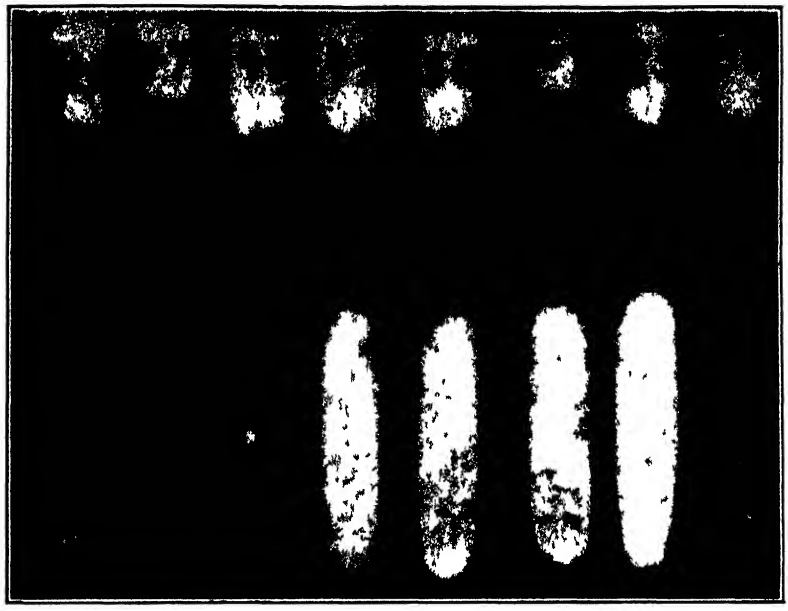


FIG. 3. Cultures of *P. omnivorum*, 15 days old, on media varying in mineral content. The importance of phosphate ion to growth of the fungus is shown by the poor growth in medium 48, which differed from 49 by omission of potassium phosphate, and medium 56 which differed from 49 by omission of phosphate ion and increase of chlorine ion.

The importance of the phosphate ion was shown again in this series. There was little growth in solutions 100 and 101, in which potassium and magnesium sulphates and chlorides respectively, were used to furnish the potassium and magnesium in the standard weights provided in potassium phosphate and magnesium sulphate of solution 104. Yet neither potassium phosphate (solutions 94, 95, 96) nor magnesium phosphate (solutions 97, 98) added individually produced appreciably better growth than in the basic solution alone (solution 93). A mixture to furnish the standard amounts of potassium and magnesium (solution 99) supported somewhat better growth, although still far inferior to that in complete formulas 104 and 70. A comparison of individual additions of different sources of phosphate, all to furnish the standard concentration of the phosphate ion, was furnished by solutions 94, 97, 102, and 103, which indicated that as the sole source of mineral nutrients, calcium phosphate was markedly superior to the potassium, magnesium, or sodium salt, as well as to the mixture of potassium and magnesium phosphate (solution 99).

The essential nature of the cations potassium, magnesium, or calcium was shown by the complete failure of growth in solution 105, which fur-

TABLE II

COMPARISON OF MINERAL NUTRIENTS, PARTICULARLY POTASSIUM AND MAGNESIUM, AS AFFECTING GROWTH OF THE FUNGUS (INCUBATED AT 24.5°–30.5°, MEAN = 27.9° C.)

Nutrient solutions			Initial reaction after autoclaving	Final results after 5 weeks		
No.	Materials added to basic formula,* gm. per liter			No. of flasks	Final reaction of media	Mean dry weight of colonies
		gm.	*pH*		*pH*	*mg.*
93	0 (basic formula only)		5.36	6	6.6	12
94	K_2HPO_4	1.35	5.35	4	4.8	14
95	K_2HPO_4	2.70	5.60	5	4.4	17
96	K_2HPO_4	6.75	5.50	6	5.2	3
97	$MgHPO_4 \cdot 3H_2O$	1.35	5.80	5	5.6	24
98	$MgHPO_4 \cdot 3H_2O$	2.70	6.15	6	6.2	16
99	K_2HPO_4	1.35	5.81	5	6.9	41
	$MgHPO_4 \cdot 3H_2O$	0.53	.....	...	.....	.....
100	K_2SO_4	1.35	4.26	4	4.8	0
	$MgSO_4 \cdot 7H_2O$	0.75	.. ...	.. ..	.. .	.. ...
101	KCl	1.15	4.92	3	4.2	7
	$MgCl_2 \cdot 6H_2O$	0.62	... ...	.	... .	.....
102	$Ca(H_2PO_4)_2 \cdot H_2O$	0.98	5.28	1	5.4	115
103	$NaH_2PO_4 \cdot H_2O$	1.07	5.39	2	5.2	5
104	K_2HPO_4	1.35	5.28	1	4.4	397
	$MgSO_4 \cdot 7H_2O$	0.75	.....	.. ..	..	.. . ...
70	K_2HPO_4	1.35	5.28	3	4.9	435
	$MgSO_4 \cdot 7H_2O$	0.75	.....	.....	... ...	....
	KCl	0.15	.....	.....	.....	... .
	$FeCl_3$	0.0015	.. ..	....	.....:	.....
105	Not added to basic formula but only:					
	$NH_4H_2PO_4$	0.89	5.56	3	4.4	0
	NH_4NO_3	0.87	.....	... ..	.....	.....
	Dextrose	40.0	.....	.. ..	.....	... .

* Basic formula 93 contains per liter (grams): ammonium nitrate 1.18, dextrose 40.0.

nished the standard concentrations of phosphate, nitrogen, and dextrose, with all other materials omitted. Sulphur or the sulphate ion also is probably essential to good growth of the fungus. Solution 99 contained the same concentrations of ions as solution 104, except for omission of the sulphate ion and slight increase in the phosphate ion, but yielded only about

one-tenth as heavy growth as the latter solution. Growth was best with the standard mixtures of mineral nutrients provided in solutions 104 and 70. The difference between growth in these two solutions was scarcely sufficient to prove that iron and chlorine ions are involved in nutrition of the fungus.

Taken together, these results indicate the importance for the nutrition of the fungus of adequate supplies of most of the mineral ions of the original BROWN formula, including phosphate, potassium, magnesium, and probably sulphate. Calcium may possibly substitute for potassium and magnesium. Iron and chlorine, if needed, are supplied in sufficient quantities by impurities in the ingredients.

SOURCES OF NITROGEN

A preliminary series to compare different sources of nitrogen was set up in May, 1930, adding to an otherwise complete basic solution (solution 25, table III) a number of organic and inorganic materials in amounts calculated to furnish the nitrogen equivalent of 2 gm. and 20 gm. of asparagin per liter. The theoretical nitrogen content of the dilute solutions was 0.424 gm., and of the more concentrated solutions, 4.24 gm., of nitrogen per liter. The basic solution was adjusted to pH 7.0, but the individual solutions made up from it were not adjusted. Each solution was used in liquid cultures, and (with 2 per cent. agar added) in additional flasks and culture-tube slants. Ammonium nitrate supported the heaviest growth. The weights of growth obtained with ammonium nitrate, glycine, asparagin, urea, potassium nitrate, and leucine varied in the order mentioned. Growth of the fungus was less in the higher concentration of most materials.

The same sources of nitrogen were compared again in the cultures summarized in table IV. The plan of this experiment was similar to that of the earlier one, except that the higher rate of nitrogen supply used here was five instead of ten times the lower rate. The second series differed also in containing di-potassium instead of tri-potassium phosphate in the basic solution, and in the adjustment of all the completed solutions to approximately pH 7.0 before autoclaving. After autoclaving, the pH ranged from 6.2 to 6.52.

The heaviest growth was obtained again with ammonium nitrate at the lower concentration, followed by peptone, urea, glycine, asparagin, potassium nitrate, leucine, and ammonium sulphate in the order mentioned. There was heavier growth with the lower concentrations of asparagin, ammonium nitrate, and ammonium sulphate and heavier growth with the higher concentrations of peptone and potassium nitrate. In this series (table IV), as in the previous one, solutions with the lower concentrations of nitrogen generally became rather acid, while corresponding solutions with higher nitrogen contents generally became less acid or somewhat alka-

TABLE III

GROWTH OF *P. OMNIVORUM* WITH VARIOUS NITROGEN SOURCES, EACH TESTED IN AMOUNTS TO FURNISH 0.424 AND 4.24 GM. OF NITROGEN PER LITER. LIQUID CULTURES WITH 40 CC. OF MEDIA PER 200-CC. FLASK (INCUBATED AT 29°–35°, MEAN = 32.4° C.)

NUTRIENT SOLUTIONS			GROWTH IN AGAR FLASKS AND SLANTS	GROWTH IN SOLUTIONS AFTER 5 WEEKS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER			No. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.			*pH*	*mg.*
25	0 (basic formula only)		Trace	.....†	.....	.....
26	Asparagin	2.0	+++	.....†	.....	.....
27	Asparagin	20.0	+++	1	8.2	286
28	Leucine	3.96	+++	0	.....	0
29	Leucine	39.6	+++	1	6.1	178
30	Glycine	2.27	++++	2	4.7	301
31	Glycine	22.7	++++	1	6.5	300
32	Peptone‡	2.65	+++	0	.....	0
33	Peptone	26.5	++++	.....†	.....	.....
34	Urea	0.91	++++	3	5.9	237
35	Urea	9.1	–	4	7.2	15
36	Ammonium nitrate	1.18	+++	3	4.5	380
37	Ammonium nitrate	11.8	++	2	5.9	115
38	Ammonium sulphate	2.00	Trace	3	3.4	32
39	Ammonium sulphate	20.0	Trace	0	.....	0
40	Potassium nitrate	3.05	+++	4	8.2	228
41	Potassium nitrate	30.5	++	5	6.9	44

* Basic formula 25 contains per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Cultures lost.

‡ Nitrogen content assumed as 16 per cent.

line. Sclerotia developed in cultures with asparagin, urea, peptone, and ammonium nitrate.

It is of interest to compare these results with those obtained by other workers. NEAL, WESTER, and GUNN (13) considered ammonium salts specifically unfavorable, since they found that ammonium nitrate and sulphate, used as sources of nitrogen in DUGGAR'S solution (in amounts to furnish approximately 12.4 gm. of nitrogen per liter), restricted growth of the root-rot fungus while growth occurred with various other nitrogen sources. These results are apparently in contradiction with our results, which instead indicate ammonium nitrate as the best source of nitrogen for the fungus. It is to be noticed, however, that NEAL and his associates used

ammonium nitrate and other nitrogen sources at concentrations nearly three times as high as those which have been shown here (table III) to be too high for best growth of the fungus.

In general, *P. omnivorum* utilized nitrogen well from organic and inorganic sources. It developed with amino acids, peptone, urea, ammonium salts, or nitrates as the source of this element.

TABLE IV

SECOND COMPARISON OF NITROGEN SOURCES FOR *PHYMATOTRICHUM*; EACH SOURCE USED IN AMOUNTS TO FURNISH 0.424 GM. OF NITROGEN PER LITER, AND ALL BUT LEUCINE, GLYCINE, AND UREA USED ALSO TO FURNISH 2.12 GM. OF NITROGEN PER LITER (INCUBATED AT 27.5°–36°, MEAN = 32.6° C.)

NUTRIENT SOLUTIONS			GROWTH IN AGAR FLASKS		GROWTH IN SOLUTIONS AFTER 37 DAYS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER		MYCELIUM	SCLEROTIA	No. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.				*pH*	*mg.*
62	0 (basic formula only)		+	–	6	6.4	7
63	Asparagin	2	+++	++++	5	5.0	391
64	Asparagin	10	++++	++	6	7.4	310
65	Leucine	3.96	++++	–	6	4.4	177
66	Glycine	2.27	++++	–	6	4.9	428
67	Urea	0.91	++++	+++	6	4.7	439
68	Peptone	2.65	++++	+	6	4.6	462
69	Peptone	13.25	++++	–	5	7.4	531
70	Ammonium nitrate	1.18	++++	+++	5	4.5	566
71	Ammonium nitrate	5.90	++++	+	6	6.9	430
72	Ammonium sulphate	2.0	++	–	6	3.2	61
73	Ammonium sulphate	10.0	+	–	6	3.9	19
74	Potassium nitrate	3.05	++++	–	3	6.8	340
75	Potassium nitrate	15.25	++++	–	2	7.4	358

* Basic formula 62 contains per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

SOURCES OF CARBON

The results of one series with carbon sources are summarized in table V. All of the materials tested supported better growth than was obtained in the checks, solution 78. Heaviest growth in 34 days was secured with the

higher concentrations of dextrose, maltose, and xylose; and definitely less growth with mannose, mannitol, lactose, and sucrose. With each source, the pH of the substratum at the end of the experiment was much lower for the more concentrated solution, and usually definitely alkaline for the less concentrated solution. Sclerotia were produced in three of the series, including the two in which heaviest growth was obtained.

TABLE V

GROWTH OF *P. OMNIVORUM* IN MEDIA CONTAINING DIFFERENT SOURCES OF CARBON, IN AMOUNTS EQUIVALENT TO DEXTROSE AT 40 AND 5 GM. PER LITER RESPECTIVELY
MEDIA ADJUSTED TO APPROXIMATELY pH 7.0 BEFORE AUTOCLAVING
(INCUBATED AT 25°–31°, MEAN = 28.2° C.)

NUTRIENT SOLUTIONS			INITIAL REACTION AFTER AUTOCLAVING	SCLEROTIA PRODUCTION IN FLASKS	GROWTH AFTER 34 DAYS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER				NO. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.	*pH*			*pH*	*mg.*
78	0 (basic formula only)		6.54	–	5	8.1	23
79	Xylose	40.0	6.51	–	4	4.8	404
80	Xylose	5.0	6.58	–	4	7.8	57
81	Dextrose	40.0	6.56	++	4	4.9	532
82	Dextrose	5.0	6.77	–	3	7.9	50
83	Mannose	40.0	6.68	++	5	5.6	289
84	Mannose	5.0	6.68	–	4	7.8	94
85	Maltose	39.0	6.69	++	5	4.6	514
86	Maltose	4.88	6.91	–	5	8.0	57
87	Sucrose	39.0	6.94	–	5	6.8	154
88	Sucrose	4.88	6.94	–	3	7.5	95
89	Lactose	39.0	6.79	–	1	6.2	180
90	Lactose	4.88	6.88	–	3	6.6	102
91	Mannitol	40.5	6.91	–	4	7.0	224
92	Mannitol	5.06	6.97	–	5	7.6	129

* Basic formula 78 contains per liter (grams): peptone 1.8, asparagin 1.8, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15.

In the series mentioned (table V), the carbon sources had been added to a solution which contained some carbon in the peptone and asparagin which supplied the nitrogen. A second series (table VI) was set up with an inorganic source of nitrogen, ammonium nitrate, so that carbon was supplied only by the various additions. The low growth in the check, as compared with solution 78 of the previous experiment, indicates that in the

earlier experiment the fungus evidently benefited from the carbon content of the organic sources of nitrogen used, and the low growth with the lactose cultures suggests that part of the growth with this sugar before may have been derived from the same cause. The trihydric alcohol, glycerin, appeared to be of no value to the fungus, although in the previous series the hexahydric alcohol, mannitol, was apparently a fairly good source of carbon, at least in the presence of the small amount of carbon already provided.

TABLE VI

GROWTH OF *P. OMNIVORUM* IN MEDIA CONTAINING DIFFERENT SOURCES OF CARBON, IN AMOUNTS EQUIVALENT TO DEXTROSE AT 40 AND AT 5 GM. PER LITER RESPECTIVELY. MEDIA ADJUSTED TO APPROXIMATELY pH 7.0 BEFORE AUTOCLAVING (INCUBATED AT 24.5°–29.5°, MEAN = 27.6° C.)

NUTRIENT SOLUTIONS			INITIAL REACTION AFTER AUTOCLAVING	GROWTH AFTER 34 DAYS		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER			NO. OF FLASKS WITH GROWTH	FINAL REACTION OF MEDIA	MEAN DRY WEIGHT OF COLONIES
		gm.	*pH*		*pH*	*mg.*
106	0 (basic formula only)		6.69	6	6.5	3
107	Dextrose	40	6.97	6	4.1	537
108	Dextrose	5.0	7.06	5	7.0	72
109	Lactose	39.0	6.97	4	5.3	41
110	Lactose	4.88	7.01	6	6.0	31
111	Glycerin	40.8	6.73	6	6.5	3
112	Glycerin	5.1	6.79	6	6.6	2
113	Corn starch	40.0	6.76	4	5.0	510
114	Corn starch	5.0	6.81	9	6.0	76
115	Corn starch, hot-air treated	40.0	6.36	6	5.5	508
116	Corn starch, hot-air treated	5.0	6.53	9	5.8	15

* Basic formula 106 contains per liter (grams): ammonium nitrate 1.18, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35.

Commercial corn starch was included in this series, in the original untreated form in solutions 113 and 114, and in solutions 115 and 116 after inactivating vitamin A by treatment in a current of air in layers 0.25 inch deep in the oven at 110° C. for 24 hours. Weights of colonies in the concentrated starch solutions, 113 and 115, were obtained after boiling for 30 minutes with 10 cc. of 0.5 N sulphuric acid, to hydrolyze the starch, prior to filtration; this treatment probably also affected the weight of the remaining fungus mats. There was no apparent difference between growth with

the untreated corn starch (solution 113) and that with the devitaminized corn starch (solution 115), each of which supported nearly as heavy colonies as did the dextrose (solution 107) which again appeared to be the best source of carbon. Later comparisons of untreated with devitaminized starches (table X) yielded somewhat different results.

It is evident from these results that the fungus can utilize carbon from starch, disaccharide and monosaccharide (pentose and hexose) sugars, and even from the polyhydric alcohol, mannitol.

Course of growth in media of high and low dextrose content

The course of growth of the fungus on some media of known different nutrient values has been studied, largely to determine the period most suitable for comparison of weights of colonies. An extensive series was set up, using solutions 15 and 18, which differed only by containing respectively 40 and 5 gm. of dextrose per liter.[4] The cultures were seeded the same day, incubated side by side on shelves in the incubator room at 26°–31° C. with a mean temperature of 27.8° C., and ten (or fewer when necessary) flasks of each set selected weekly at random and removed for weighing.

After 2 weeks, the colonies in both media consisted of fairly light growth with concentric rings alternately of hyaline, submerged mycelium and of white, floating mycelium. After 3 weeks the entire surfaces of the solution 15 (4 per cent. dextrose) colonies were filled in with abundant mycelial growth, portions of which had already developed a light buff color. With solution 18, however, the concentric-ring growth was unobscured by any further development and remained approximately the same during the rest of the experiment. At the end of the third week, the colonies in solution 18 averaged only 157 mg., and the weights of colonies decreased progressively thereafter. In solution 15, the colonies continued to increase in weight through the fifth week, reaching then the peak weight of 558 mg., after which the weights of these colonies decreased each succeeding week also.

The average weights of colonies harvested after the various periods of incubation are given graphically in figure 4. An idea of how closely representative such averages were of the entire groups of cultures harvested at these times may be obtained from the standard deviations and coefficients of variation of these averages. For solution 15, the standard deviations were 1.8, 21.7, 38.8, and 47.8 mg. respectively, for the first 4 weeks of the experiment, and fell thereafter progressively to 37.6, 20.9, 14.7, 4.4, and 5.6 mg. However, the coefficients of variation decreased steadily from the first: 46, 34, 14, 9, 7, 4, 3, 1, and 1 per cent. By the third week the colo-

[4] In addition to dextrose, solutions 15 and 18 contained per liter (grams): peptone 2, asparagin 2, KCl 0.15, $MgSO_4 \cdot 7H_2O$ 0.75, K_2PO_4 1.35, $FeCl_3$ 0.0015.

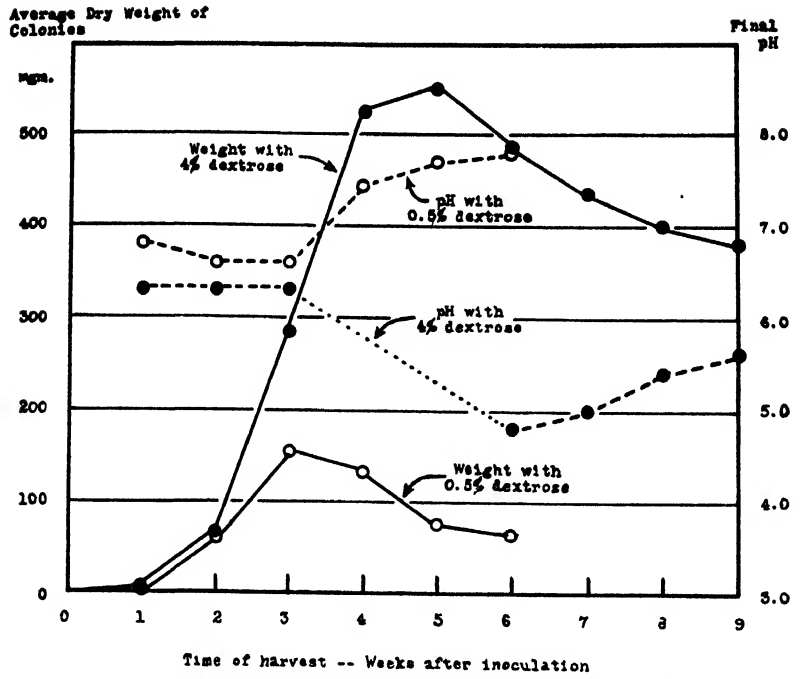


FIG. 4. Growth curves of *P. omnivorum* on solution 15, containing 4 per cent. dextrose, and on solution 18, containing 0.5 per cent. dextrose. Note rise in pH which occurred with cessation of increase in growth.

nies had grown to oven-dry weights of 230.3–357.5 mg., with the standard deviation of 38.8 mg. amounting to 14 per cent. of the mean weight of 286 mg. Although the colonies harvested 2 weeks later ranged from 496.8 to 618.6 mg. in weight, so many fell near the average weight that the standard deviation was only 37.6 mg. and the coefficient of variation only 7 per cent. In solution 18, the standard deviations decreased similarly after the third week, while the coefficients of variation decreased after the second week. With both media, the considerable variability of the younger colonies was thus quantitatively shown to be gradually minimized as growth continued, and the colonies were exposed to similarly limiting factors such as size of flasks, nutrient supply, temperature, etc.

With this information it would appear that, for a single representative harvest of the present series, a sample taken possibly at the end of the fifth week would yield more accurate results than an earlier sample. In such a single sample, on the other hand, growth might already have reached its peak and begun to decrease, as would be true here with solution 18. These considerations lead to two conclusions. First, in comparing weights of colonies in different media, as in tables I to VI, it is to be remembered that the

particular weights shown may represent samples from ascending, peak, or descending portions of the growth curves, and that these figures do not therefore represent specific indexes of the nutrient value of the various media, but merely indicate their probable relative value. Second, since samples after any particular period of incubation appear inadequate for careful comparison of substrata, harvests should be made when possible after 3, 4, and 5 weeks rather than after merely one period. This has been done with certain of the experiments to be discussed in a following section, and also in the study of plant juices as presented elsewhere (6).

Another growth experiment was run later, using three solutions with respectively 4, 2, and 0.5 per cent. of dextrose, and with ammonium nitrate as the source of nitrogen.[5] The cultures were incubated at 22°–32 C., with a mean temperature of 27.3° C. The weights of fungus colonies from five flasks of each solution, and of reducing sugars remaining in the culture solutions, were determined weekly, and are shown graphically in figure 5.

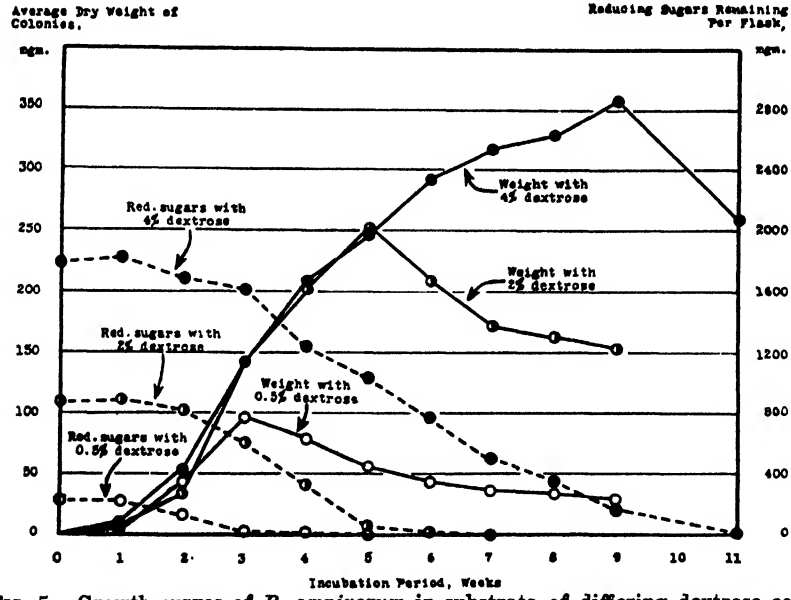


FIG. 5. Growth curves of *P. omnivorum* in substrata of differing dextrose content, showing weekly weights of fungus colonies, and weights of reducing sugars remaining in culture solutions. Note increases in colony weight until dextrose was almost completely exhausted.

In this second experiment growth was much slower than in the former series, the peak with 4 per cent. dextrose coming after 9 instead of after 5 weeks, and that with 2 per cent. dextrose after 5 instead of after 3 weeks.

[5] In addition to the dextrose, these solutions contained per liter (grams): ammonium nitrate 2.12, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

Colonies increased in weight in all three media at approximately the same rate, and apparently without regard to the differences in original concentration of dextrose; and this increase continued with each medium until most of the dextrose supply was exhausted. Thus with 2 per cent. dextrose solution, growth for 5 weeks was almost precisely parallel to that with 4 per cent. After 5 weeks, however, the dextrose supply had fallen to about 67 mg. per flask, and in the next harvest the fungus colonies showed a sharp decrease in weight. The supply of dextrose apparently became the limiting factor in growth of the fungus only after the dextrose concentration fell to around one-tenth of 1 per cent.

The decrease in weight of colonies in the descending portions of the curves must have been due in part to respiratory activity, and in part to autolytic decomposition of the older cells of the mycelium. This autolysis evidently did not destroy the viability of the cultures during the period of this experiment, since successful transfers to agar slants were made after 9 weeks from the cultures in the 2 and in the 0.5 per cent. dextrose solutions.

Taken together, the various studies on the relation of the root-rot fungus to different concentrations of dextrose illustrate the wide variations of conditions suitable for the fungus. It grew well in solutions containing about 16 per cent. of dextrose (table X), yet in media containing respectively 0.5, 2, and 4 per cent. of dextrose its rate of growth was constant until the dextrose content in the various solutions had fallen successively to a small fraction of 1 per cent. The fact that growth meanwhile continued in the media which contained the higher carbon contents indicated that further growth in the more dilute solutions was in fact prevented by the exhaustion of the carbohydrate rather than by the simultaneous increase in acidity. As shown graphically in figure 4, media became increasingly acid during active growth of the colonies. With exhaustion of the dextrose the colonies ceased to increase in weight, autolysis of the mycelium became predominant over growth, and the reaction of media shifted toward alkalinity. This relation was noted also in the second course of growth experiment as well as in other experiments. Media were generally more acid at the end of 3 or 4 weeks if the weights were at the maximum at that time, and more alkaline by the fifth week if the colonies meanwhile decreased in weight.

The explanation of this sequence is presumably the same as with most fungi. It is well established that fungi produce various organic acids, such acids as oxalic, citric, acetic, and kojic (**11**) having been identified as the products of fungus metabolism. The production of acid is generally linked with active growth in the presence of carbohydrates, and acids produced during growth may be utilized after the carbohydrate supply is exhausted. When this occurs, autolytic decomposition of the mycelium becomes the chief factor in further change of media, which become alkaline perhaps

mostly from evolution of ammonia. In alkaline media of this sort the basic radicals set free presumably combine with the carbon dioxide of respiration to produce bicarbonates, which have been thought by recent workers (**14**, **10**) to be the general cause of "staling" in old culture media.

Hydrogen-ion concentration of culture solutions

Earlier work with the root-rot fungus, particularly in its growth in soil and on cotton plants (**16**, **4**), has shown that it is rather sensitive to acid reactions. In solutions acidified with hydrochloric, sulphuric, and acetic acids respectively, growth was inhibited by reactions of pH 4.1 and checked greatly in media more acid than about pH 6 (**16**). In the present studies, however, good growth was frequently obtained in media more acid than pH 5. This acidity was due to the presence of mono-potassium phosphate, of amino acids supplied as sources of nitrogen, or of acid products of the fungus metabolism. It has therefore been of interest to study the growth of the fungus in media made acid by additions of phosphoric acid.

In a preliminary series, cultures were grown for 5 weeks on media adjusted from pH 3.1 to 8.7 by additions of phosphoric acid and potassium

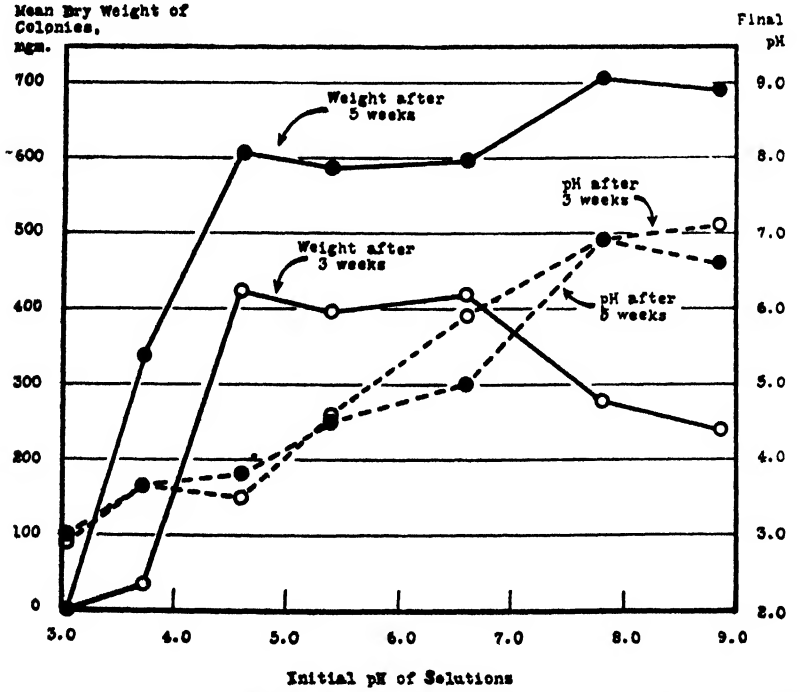


Fig. 6. Growth of *P. omnivorum* in solutions made acid by addition of phosphoric acid, and alkaline by addition of potassium hydroxide, respectively; and pH of media determined after initial aseptic adjusting and after respective periods of growth.

hydroxide respectively. Growth occurred in all except the most acid solution, which remained at about pH 3.0. Very slight growth, averaging only 41 mg., was obtained in the next most acid solution, which had been autoclaved after addition of the acid and had a final pH of 3.2. The other media, with initial values ranging from pH 4.1 to 8.7, and final values of pH 3.5 to 6.3, all supported good growth, the average weights of colonies in these media coming between 478 and 533 mg. Heaviest growth was in the two more alkaline substrata.

A more extensive series was set up later, adding (aseptically without further autoclaving) phosphoric acid for the more acid solutions, and potassium hydroxide for the more alkaline media, to nutrient solution 81 (table V). Five colonies in each solution were weighed after 3 weeks, and another five colonies after 5 weeks. The results are shown graphically in figure 6. Growth was inhibited in the most acid solution, at pH 3, but not in the most alkaline. Best growth was again in the two most alkaline solutions, which were adjusted originally to pH 7.81 and 8.85 respectively, but shifted approximately to neutrality by the end of 3 weeks.

These series indicate that the fungus may be somewhat less sensitive to phosphoric acid than to the acids used in the previous study (**16**). It is well known that the toxicity of acid culture solutions is influenced not alone by the hydrogen-ion concentration produced, but also by the specific acids present. Further direct comparison of the effect of different acids on the growth of *P. omnivorum* is planned.

Composition of fungus hyphae, and preliminary test for staling products

Observations on the root-rot fungus in culture solutions, in soil chambers, and on the roots of plants have shown that individual vegetative cells of the mycelium deteriorate comparatively rapidly as compared with the duration of the colony as a whole. Taubenhaus and Killough (**17**) described the breaking down of older cells of the hyphae and the characteristic production of deep brown droplets over the surfaces of colonies. In numerous inoculation experiments, it was found that the fungus did not remain viable on infected roots for many weeks after the inception of particular portions of lesions (**15**, p. 769–773). The question arose as to whether this deterioration of mycelium of the fungus under a wide variety of conditions, in some cases obviously from exhaustion of nutrients, might not be due partly to production by the fungus of materials toxic to its own further development, in other words, of "staling products."

A preliminary experiment was set up with 30 large, 1-liter flasks, each containing 200 cc. of culture solution 123 (table VII). Cultures were allowed to grow for 33 days; the solutions were then poured off and the colo-

TABLE VII

COMPOSITION OF *P. OMNIVORUM* MYCELIUM FROM 33-DAY-OLD COLONIES GROWN ON SOLUTION 123* AT 22°–32°, MEAN = 26.9° C. (ANALYSES ON TOTAL OF 13.8 GM. OVEN-DRY MYCELIUM)

	PERCENTAGE
Protein (N × 6.25)	29.25
Fat	4.02
Crude fiber	23.90
Nitrogen-free extract	35.62
Water	1.35
Ash	5.86
Ash included:	
Phosphoric acid (P_2O_5)	2.74
Potash (K_2O)	1.39
Lime (CaO)	0.12
Magnesia (MgO)	0.35
Insoluble ash	1.07

* Solution 123 contains per liter (grams): peptone 4.2, dextrose 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

nies dried for analysis. It may be noted (table VII) that the protein content of these 33-day-old colonies was higher than that of 21-day-old fungus mats grown in solution 81 and analyzed previously. With the latter, colonies with oven-dry weights from 331 to 426 mg. showed protein contents from 24.1 to 15.0 per cent. respectively, the higher percentages and higher actual amounts of protein coming in the lighter colonies.

The clear, reddish brown liquid poured off from the flasks still contained 3.98 gm. of dextrose per 200 cc., approximately half the original amount. This proportionate utilization of dextrose in 5 weeks compares well with that shown in the growth curves (fig. 5) for the same period, and suggests that the colonies in these large flasks were probably still growing actively.

Some of this old fluid was transferred with aseptic precautions to sterile flasks, and some to flasks containing previously sterilized, new synthetic culture solution. Duplicate series were run with autoclaved portions of the old fluid (table VIII). The fungus grew well in this old fluid alone, and mixtures of the fluid with the fresh solution made a nutrient medium which yielded heavier growth than the synthetic medium alone. These results therefore furnished no indication of formation of staling products by the root-rot fungus, at least up to a 33-day stage of growth under the conditions of this experiment. Inasmuch as many fungi are able to utilize the acids produced during their early development, and are said to be impeded only by too high concentrations of these acids or by the final alkaline

bicarbonates produced in autolytic decomposition, it will be of interest later to test the possible toxicity of culture media in which definite alkalinity has developed from autolytic decomposition of the fungus.

TABLE VIII

GROWTH OF *P. OMNIVORUM* ON OLD CULTURE LIQUID ON WHICH THE FUNGUS HAD PREVIOUSLY GROWN FOR 33 DAYS (SEE TABLE VII); 20 CC. OF MEDIA PER 150 CC. ERLENMEYER FLASKS (INCUBATED AT 23.5°–28.5°, MEAN = 26.0° C.)

COMPOSITION OF MEDIA	RESULTS AFTER 3 WEEKS			RESULTS AFTER 5 WEEKS		
	FINAL REACTION OF MEDIA	NO. OF FLASKS	MEAN DRY WEIGHT OF COLONIES	FINAL REACTION OF MEDIA	NO. OF FLASKS	MEAN DRY WEIGHT OF COLONIES
	pH		*mg.*	*pH*		*mg.*
Old fluid, aseptically removed and unheated, alone	6.4	1	76	5.6	2	118
Old fluid, autoclaved, alone	6.6	3	53	6.0	3	88
Unheated fluid, 5 cc. plus solution 70*, 15 cc	7.2	3	129	5.1	3	239
Autoclaved fluid, 5 cc. plus solution 70, 15 cc	7.4	3	116	5.2	3	248
Distilled water, 5 cc. plus solution 70, 15 cc	7.2	3	156	5.2	3	162

* For composition of solution 70, see table IV.

POSSIBLE ACCESSORY GROWTH-PROMOTING SUBSTANCES

In studies on growth of the root-rot fungus in plant juices (**6**), it has been found that addition to synthetic media of small amounts of expressed plant juices yielded rather large increases in fungus growth. This suggested that some accessory growth-promoting substances, perhaps similar to a vitamin, might be involved in nutrition of the fungus. Vitamins or similar substances have been found necessary to growth of certain other fungi. For instance, WILLAMAN (**18**) concluded that *Sclerotinia cinerea* (now *S. americana*) required an accessory material of properties similar to vitamin B. FARRIES and BELL (**7**) found that while many fungi grew on the simplified BROWN's medium utilized also in the present study (solution 4), *Nematospora coryli, N. gossypii,* and *Spermophthora gossypii* required in addition some active accessory material which was apparently present as an impurity in various natural protein sources. MARLOTH (**10**) has

TABLE IX

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA ALONE AND WITH ADDITION OF CARROT JUICE MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 23°–32°, MEAN = 26.7° C.)

NUTRIENT SOLUTIONS*			INCUBATION PERIOD	MEAN DRY WEIGHT OF COLONIES, FROM SOLUTIONS WITH FOLLOWING ADDITIONS PER 50 CC.†	
No.	NITROGEN SOURCES	CARBON SOURCES		NONE (CHECK)	CARROT JUICE (2.5 CC.)
	gm.	*gm.*	*weeks*	*mg.*	*mg.*
68	Peptone 2.65	Dextrose 40	3	85	238
			4	217	361
			5	312	520
70	Ammonium nitrate 1.18	Dextrose 40	3	125	286
			4	235	562
			5	351	607
74	Potassium nitrate 3.05	Dextrose 40	3	71	230
			4	216	437
			5	211	530
81	Asparagin 1.8 and peptone 1.8	Dextrose 40	3	238	358
			4	498	662
			5	660	697
87	Asparagin 1.8 and peptone 1.8	Sucrose 39	3	345	387
			4	516	608
			5	715	780
124	Asparagin 1.8 and peptone 1.8	Dextrose 20 and sucrose 19.5	3	161	453
			4	457	644
			5	584	704

* For complete formulas of the solutions, see tables IV and V.

† Mean values for three flasks per series after 3 and 4 weeks, and for four flasks per series after 5 weeks.

noted that growth of *Penicillium digitatum* was so markedly accelerated by addition of a small amount of orange juice to synthetic media as to suggest that this fungus may require a vitamin occurring in citrus fruits for its growth.

Some experiments have been run to test the possibility of accessory materials being involved in nutrition of *P. omnivorum*. A first series was prepared from a uniform mineral base with the nitrogen and carbon sources varied as indicated in table IX. The same weights of carbon were

used in all, but the nitrogen content of solutions 68, 70, and 74 was only a little more than half that of the other three media. Each solution was used alone and also with the addition of 2.5 cc. of carrot juice (containing 0.17 gm. of dry material) per 50 cc. of solution (containing about 2.2 gm. of solid matter). The carrot juice was a portion of that prepared for other work (**6**, table VI) and had been expressed, autoclaved, filtered to remove the coagulum precipitated by the autoclaving, and re-autoclaved. Analyses showed that the 2.5 cc. of juice (1) added only 0.117 gm. of sugar to 2.0 gm. already present in 50 cc. of solution 68, for example; (2) added only 2.5 mg. of nitrogen to 21.2 mg. already provided; (3) added 6.0 mg. of potassium to 34.0 mg. provided; (4) added 0.5 mg. of phosphorus to 12.0 mg. provided; and (5) added 0.3 mg. of magnesium to 3.7 mg. provided.

Increases in growth were obtained with the carrot additions to all the solutions. The increases were markedly greater, both proportionately and absolutely, however, with solutions 68, 70, and 74 than with the other three solutions. The heavy growth in solutions 81, 87, and 124, even when used alone, was apparently due to the additional nitrogen and possibly to the particular sources of the nitrogen provided. This would suggest that increases following addition of carrot juice also may have been due to the nitrogen supplied in the juice. On the other hand, the asparagin used may have contained accessory growth-promoting substances as impurities and may have supplied these materials to the fungus.

The possibility that other ingredients of the formula might be carrying impurities influencing the results was considered in another experiment, in which ammonium nitrate was used as the source of nitrogen for all media. The growth obtained (table X) was similar with dextrose from an old lot and with dextrose from a new lot at 40 to 160 gm. per liter. Omission of ferric chloride and potassium chloride resulted in a slight decrease in growth, and a similar decrease occurred when all mineral ingredients were used at double strength. Accessory growth-promoting materials were therefore probably not present as impurities in the dextrose or mineral portions of the media; otherwise increasing the proportions of these ingredients should have resulted in greater accumulation of such impurities and greater growth.

The last two solutions of this series contained untreated and hot-air treated corn starch prepared in the same way as that tested earlier (table VI). In the present experiment both starches were dextrinized, prior to use in the culture solutions, by boiling 20-gm. lots for 150 minutes in 300 cc. water plus 25 cc. of 0.2 N hydrochloric acid, and then filtering. This hydrolysis did not transform much starch to sugar, and the larger amount of sugar was with the hot-air treated starch. Nevertheless more rapid and heavier growth occurred in the other solution. This agreed with other ob-

TABLE X

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA WITH VARIATIONS IN DEXTROSE AND MINERAL SUPPLY, AND WITH PLAIN AS COMPARED WITH HOT-AIR TREATED STARCH MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 22°–30.5°, MEAN = 26.0° C.)

NUTRIENT SOLUTIONS		SUGARS IN 50 CC. OF AUTOCLAVED SOLUTIONS		MEAN DRY WEIGHT OF COLONIES†		
No.	MATERIALS ADDED TO BASIC FORMULA,* GM. PER LITER	TOTAL	REDUCING	3 WEEKS	4 WEEKS	5 WEEKS
	gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*
70	Dextrose 40	1.6864	1.6864	190	267	467
70B	Dextrose‡ 40	1.6864	1.6812	202	283	351
125	Dextrose 80	.....	....	208	302	385
126	Dextrose 160	.....	.....	63	241	441
104	Dextrose 40 ($FeCl_3$ and KCl omitted)	.....	.....	156	237	316
129	Dextrose 40 (and all mineral ingredients doubled)	.....	... .	103	248	306
127	Corn starch, dextrinized, 40	0.1755	0.1715	249	419	578
128	Corn starch, hot-air treated then dextrinized, 40	0.3397	0.2842	43	57	348

* Basic formula contains per liter (grams): ammonium nitrate 1.18, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Mean values for three flasks per series after 3 and 4 weeks, and for four flasks per series after 5 weeks.

‡ Dextrose for 70B came from an old bottle of dextrose as used in previous experiments, the dextrose for all the other solutions being from a new lot.

servations with series prepared from unhydrolyzed starches, and thus not available for weighing. Heavier growth with corn starch that had not been given hot-air treatment (a difference not always obtained, see table VI) may have been due to the presence in untreated starch of vitamin A or some other material inactivated by the hot-air treatment, or to the presence in the hot-air treated starch of some product detrimental to growth of the fungus.

The uncertain nature of these results suggested a further test with known vitamin sources. Two general basic formulas were used (table XI), the first with solutions 63, 70, and 132, containing most of the ingredients in the standard concentration, while the formula used with solutions 130 and 131 contained much higher amounts of nitrogen and of minerals, to approximate the contents of complete carrot juice as shown by analysis.

Each basic formula was used with the various nitrogen sources indicated in table XI, and each complete solution was then used alone and with the addition respectively of three possible sources of vitamins. To one set of cultures, 2.5 cc. per flask of carrot juice were added, as in the earlier experiment (table IX); to another set, 1 drop (0.03 cc.) per flask of cod-liver oil[6] was added as a possible source of vitamin A; and to another series, 2 drops (0.087 cc.) per flask of rice-bran extract[7] as a possible source of vitamin B.

TABLE XI

GROWTH OF *P. OMNIVORUM* IN SYNTHETIC MEDIA ALONE, AND WITH ADDITIONS OF SMALL QUANTITIES OF VITAMIN SOURCES. MEDIA ADJUSTED ORIGINALLY TO APPROXIMATELY pH 7.0 (INCUBATED AT 29.5°–35.0°, MEAN = 32.9° C.)

NUTRIENT SOLUTIONS		INCUBATION PERIOD	MEAN DRY WEIGHT OF COLONIES, FROM SOLUTIONS WITH FOLLOWING ADDITIONS PER 50 CC.			
No.	MATERIALS ADDED TO BASIC FORMULA* † (GM. PER LITER)		NONE (CHECK)	CARROT JUICE (2.5 CC.)	COD-LIVER OIL (1 DROP)	RICE-BRAN EXTRACT (2 DROPS)
	gm.	*weeks*	*mg.*	*mg.*	*mg.*	*mg.*
63	Asparagin ..... 2.0 *	3	323	438	314	323
		4	498	629	522	492
		5	574	587	565	548
70	Ammonium nitrate 1.18*	3	244	429	347	280
		4	372	570	511	384
		5	503	529	589	512
132	Ammonium nitrate 1.18 and asparagin 2.0 *	3	365	586	490	483
		4	569	703	486	561
		5	570	560	583	513
130	Ammonium nitrate 1.41†	3	60	275	36	77
		4	87	464	44	258
		5	188	608	188	359
131	Ammonium nitrate 0.32 and asparagin 1.8†	3	25	114	19	39
		4	52	227	39	69
		5	81	307	81	103

* Basic formula for solutions 63, 70, and 132 contained per liter (grams): dextrose 40, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.35, KCl 0.15, $FeCl_3$ 0.0015.

† Basic formula for solutions 130 and 131 was calculated to contain with the nitrogen additions the approximate materials included in complete carrot juice and contained per liter (grams): dextrose 24.3, sucrose 22.4, $Ca(NO_3)_2 \cdot 4H_2O$ 0.93, KNO_3 2.95, K_2HPO_4 1.202, KCl 1.35, $MgSO_4 \cdot 7H_2O$ 1.16, $FeCl_3$ 0.0015.

[6] Cod-liver oil used was from a newly opened bottle from E. R. Squibb & Sons, and was labeled to contain not less than 70,000 U.S.P. units of vitamin A and not less than 2,600 units of vitamin D per 100 gm.

[7] Rice-bran extract prepared by soaking 10 gm. of commercial rice bran in 100 cc. of cold water for 2 hours, with occasional stirring, and then filtering to remove the bran.

All media were autoclaved after the various additions, so that thermolabile materials were presumably inactivated.

A question raised by the earlier results was whether asparagin might not have carried some accessory materials as impurities. In the present experiment, solution 63 contained as asparagin the same weight of nitrogen that was contained in solution 70 as ammonium nitrate. Growth was definitely more rapid in the former, and also attained a somewhat greater weight. On the other hand, solution 131 contained the same materials as 130 except that part of the nitrogen was supplied as asparagin instead of as ammonium nitrate, and it proved a poorer substratum for the fungus than solution 130.

The addition of cod-liver oil produced increased growth only with solution 70; with the rice-bran extract, growth increased definitely with solution 130, increased slightly in 131, and decreased in solutions 63 and 132. On the other hand, addition of carrot juice resulted in marked increases in the rate and final extent of growth with all five solutions. With the more concentrated synthetic bases, solutions 130 and 131, the addition of carrot juice appeared particularly valuable, apparently by obviating in some way some harmful effect from these concentrated media. In the concentrations tested here, vitamin A from cod-liver oil and vitamin B from rice-bran extract were apparently of little nutritive value to *Phymatotrichum omnivorum*, and probably neither of these materials is the basis for the increase in growth obtained from addition of carrot juice to synthetic media.

Synthetic media as influencing sclerotia production

For more than 2 years, series of 2 per cent. agar flask cultures have been maintained uninterruptedly to provide sclerotial material for use as inoculum in numerous lines of work. Each series included media prepared by two or three different formulas, and these were replicated periodically, usually to a total of 20–40 flask cultures of each substratum, until the more dependable medium for production of sclerotia was selected. An early series was made up from a basic formula similar to solution 48 (table I), except that corn starch was used instead of dextrose. Sclerotia developed profusely in 2 weeks in media to which mono-, di-, and tri-potassium phosphate respectively had been added at 1.35 gm. per liter, and which ranged in reaction from pH 4.6 to 8.4; but in a fourth series with no phosphate, sclerotia developed only after 4 weeks, and then in small quantities. The di-potassium phosphate medium (formula 44) was compared with media in which respectively the quantity of potassium phosphate was doubled, and dextrose was substituted for the starch; and much heavier sclerotia production appeared with formula 44. Formula 44, in which nitrogen was supplied partly as the relatively expensive asparagin, was then compared

with 76, in which all the nitrogen was supplied in 4.185 gm. of peptone per liter, and at least equally heavy production of sclerotia was obtained with the latter formula.

Formula 76 was found later to be much superior to media containing hot-air treated corn starch or dextrose instead of the untreated commercial corn starch. It was then compared with a more concentrated medium, 119, which included both dextrose and corn starch as sources of carbon, and both peptone and ammonium nitrate as sources of nitrogen. Formula 119 included the ingredients of solution 70 (table IV) plus 4.185 gm. of peptone and 40 gm. of dextrose per liter. In series repeated for more than 6 months, both media proved excellent for the production of sclerotia, but sclerotia developed almost invariably much earlier and frequently more profusely with 119.

Recent modifications include omission of the small amounts of KCl and $FeCl_3$, substitution of commercial sucrose for the dextrose, and rounding-off of the quantities of the other ingredients. The simplified formula 134[8] has now been tested for 10 months, in eight successive series. In all of seventy flask cultures with this substratum an abundance of sclerotia was produced, the masses extending around the flasks, reaching an average height of 30 mm. above the surface of the substratum, and often a thickness of 4–8 mm. This substratum, while doubtless susceptible of further improvement, can be recommended as useful for production of sclerotia. Omitting the corn starch produced a clearer material (formula 135), which has proved useful for general culture purposes with a number of other fungi previously carried on potato-dextrose agar, but has yielded only small quantities of *Phymatotrichum* sclerotia.

Comparative study of sclerotia production on the synthetic substrata just described, and also in the media included in previous sections of this paper, not only has made possible the development of dependable media for sclerotia production but also has furnished an insight into conditions which influence the inception of this stage of the life history, at least under these cultural conditions. It appears clear that nutritive conditions highly favorable for vegetative growth are also favorable for development of sclerotia. Nutritive and temperature conditions which impede vegetative growth apparently do not stimulate sclerotia formation, which is instead correlated with vigorous vegetative development.

Production of sclerotia in synthetic media in the laboratory is obviously not directly comparable with the development of sclerotia under natural conditions in the field. Yet it appears probable that production of sclerotia

[8] Formula 134, used in cultures for sclerotia production, includes per liter (grams): peptone 4.2, ammonium nitrate 1.2, corn starch (commercial) 40.0, sucrose (commercial) 40.0, $MgSO_4 \cdot 7H_2O$ 0.75, K_2HPO_4 1.5, agar 20.0.

in the field also is correlated with conditions of rapid growth and abundant nutrient supply for the root-rot fungus, rather than with conditions of retarded growth and insufficient nutrients. It would follow that treatments which militate against rapid and free vegetative growth of the fungus in the field might have a further desirable effect in discouraging production of these long-lived resting bodies of the fungus.

Discussion

From comparative studies of growth of *Phymatotrichum omnivorum* on numerous synthetic culture solutions, it is found that the nutrient requirements of the fungus are not very exacting. This accords with the wide range of host plants, including possibly the majority of gymnosperms and dicotyledonous plants, which are attacked by this fungus. It is well known, however, that many fungi with restricted host ranges can nevertheless be cultured on similarly varied synthetic media. Within the limits of the nutrient variables considered in the present work, therefore, the extent of nutrient conditions under which the root-rot fungus can be grown would appear neither particularly unusual nor likely to be of much significance in relation to the host range. This conclusion agrees with results obtained in the concurrent study of growth of the fungus in plant juices (**6**), which showed that immune monocotyledonous plants were protected, not by lack of necessary nutrient materials, but by the presence of some specifically toxic material.

Summary

1. Mineral ions utilized by the root-rot fungus, *Phymatotrichum omnivorum,* were phosphate, potassium, magnesium, and probably sulphate. Calcium, iron, and chlorine, if needed, were supplied in sufficient quantities by impurities in the ingredients.

2. Nitrogen was utilized equally well from organic sources, such as amino acids, peptone, and urea, and from inorganic ammonium and nitrate salts. Ammonium nitrate was frequently the best source of nitrogen.

3. As sources of carbon, the fungus utilized pentose and hexose monosaccharide sugars, disaccharide sugars, starch, and to a lesser extent the polyhydric alcohol mannitol.

4. The fungus grew at the same rate in media containing various concentrations of dextrose. Colonies increased in weight and the media became increasingly acid until the dextrose content of the solutions fell to a fraction of 1 per cent. After exhaustion of the dextrose, weight of colonies decreased and the reaction of the media changed rapidly toward alkalinity.

5. In media adjusted to various reactions by phosphoric acid and potassium hydroxide respectively, best growth was obtained in the more

alkaline solutions. Growth was inhibited at approximately pH 3, but good growth occurred at pH 3.7.

6. No indication of staling products was obtained in tests of substrata in which the fungus had grown for 33 days.

7. Small quantities of carrot juice added to synthetic media resulted in disproportionately large increases in growth of the fungus, and suggested that some accessory growth-promoting material might be involved. In one experiment, addition of carrot juice to solutions containing asparagin as a source of nitrogen resulted in smaller increases in growth than were obtained with carrot juice additions to other media. Addition of cod-liver oil (as a source of vitamin A) and of rice-bran extract (as a source of vitamin B) to culture solutions produced insignificant changes in growth of the fungus. The basis of increased growth with the juice addition may be the specific organic nitrogen source provided in the juice, some accessory growth-promoting material, or some other factor.

8. Sclerotia developed most abundantly in media most suited for rapid and abundant vegetative growth. The formula of a nutrient medium developed specifically for sclerotia production is given.

9. The variety of nutrient conditions suitable for growth of this fungus accords with the wide host range of the fungus. Limitation of hosts of root rot, however, has already been shown to be determined apparently by the presence of toxic materials in immune monocotyledonous plants, rather than by the lack of nutrients in such plants.

The writers take pleasure in expressing their appreciation of the advice of Dr. G. S. FRAPS, Chief of the Division of Chemistry, in connection with various phases of the work.

AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS

LITERATURE CITED

1. ATKINSON, G. F. Method for obtaining pure cultures of Pammel's fungus of Texas root rot of cotton. Bot. Gaz. **18**: 16–19. 1893.
2. BROWN, W. Studies in the genus *Fusarium*. II. An analysis of factors which determine the growth-forms of certain strains. Ann. Bot. **39**: 373–408. 1925.
3. DUGGAR, B. M. The Texas root-rot fungus and its conidial stage. Ann. Missouri Bot. Gard. **3**: 11–23. 1916.
4. EZEKIEL, WALTER N., TAUBENHAUS, J. J., and CARLYLE, E. C. Soil-reaction effects on *Phymatotrichum* root rot. Phytopathology **20**: 803–815. 1930.

5. ————, ————, and FUDGE, J. F. Nutritional studies on *Phymatotrichum omnivorum*. (Abst.) Phytopathology **21**: 120. 1931.
6. ————, ————, and ————. Growth of *Phymatotrichum omnivorum* in plant juices as correlated with resistance of plants to root rot. Phytopathology **22**: 459–474. 1932.
7. FARRIES, E. H. M., and BELL, A. F. On the metabolism of *Nematospora gossypii* and related fungi, with special reference to the source of nitrogen. Ann. Bot. **44**: 423–455. 1930.
8. KING, C. J., and LOOMIS, H. F. Cotton root-rot investigations in Arizona. Jour. Agr. Res. **39**: 199–221. 1929.
9. ————, and ————. Further studies of cotton root rot in Arizona, with a description of a sclerotium stage of the fungus. Jour. Agr. Res. **39**: 641–676. 1929.
10. MARLOTH, R. H. The influence of hydrogen-ion concentration and of sodium bicarbonate and related substances on *Penicillium italicum* and *P. digitatum*. Phytopathology **21**: 169–198. 1931.
11. MAY, O. E., MOYER, A. J., WELLS, P. A., and HERRICK, H. T. The production of kojic acid by *Aspergillus flavus*. Jour. Amer. Chem. Soc. **53**: 774–782. 1931.
12. NEAL, D. C. The occurrence of viable cotton root-rot sclerotia in nature. Science n. s. **70**: 409–410. 1929.
13. ————, WESTER, R. E., and GUNN, K. C. Treatment of cotton root-rot with ammonia. Science n. s. **75**: 139–140. 1932.
14. PRATT, C. A. The staling of fungal cultures. II. The alkaline metabolic products and their effect on the growth of fungal spores. Ann. Bot. **38**: 599–615. 1924.
15. TAUBENHAUS, J. J., and EZEKIEL, W. N. Studies on the overwintering of *Phymatotrichum* root rot. Phytopathology **20**: 761–785. 1930.
16. ————, ————, and KILLOUGH, D. T. Relation of cotton root rot and *Fusarium* wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bull. 389. 1928.
17. ————, and KILLOUGH, D. T. Texas root rot of cotton and methods of its control. Texas Agr. Exp. Sta. Bull. 307. 1923.
18. WILLAMAN, J. J. The function of vitamines in the metabolism of *Sclerotinia cinerea*. Jour. Amer. Chem. Soc. **42**: 549–585. 1920.

EFFECTS OF HUMIDITY ON METABOLISM IN TOMATO AND APPLE[1]

G. T. NIGHTINGALE AND J. W. MITCHELL

(WITH ONE FIGURE)

The literature on effects of specific factors of environment includes reports which are often contradictory in greater or less degree. The reason sometimes would seem apparent. In considering the results obtained with respect to plant appearance, anatomy, or composition, the causal factor has often been assumed by the investigator to be solely that which was varied experimentally. Perhaps the most that may be said is that when one or another factor of environment is apparently dominant, as temperature, for example, metabolism and growth of the plant may under that temperature and specific total-environment show certain trends; trends, however, which can be accentuated or masked by modification of nutrition or carbon dioxide supply, light intensity, or other factors (**22**).

The quality of growth of plants, however, is always the expression of the specific character of their metabolic processes involving all assimilated products (**15**), and in part unassimilated materials (**8, 12, 14, 16**), guided and often qualitatively and quantitatively directed by the factors of the environment. A shift in one or more of these factors is reflected in the type of growth of the plant. With these points in mind, a study was made of the growth responses and accompanying chemical changes which were found to take place in tomato plants subjected to an environment in which humidity was the single variable factor for a given nutrient treatment. The nitrate reducing power of these plants, together with microscopic observations, are reported in detail by ECKERSON (**4**), although frequent reference to her work will be made in the following pages.

PART I. TOMATO

Experimental methods

Recent papers (**11, 19, 20**) record the results of experiments in which plants were grown at the same relative humidity but at different temperatures, the lowest being 45° and the highest 95° F. The rate of evaporation from leaves must be closely related to the vapor pressure deficit of the

[1] Through the courtesy of the University of Chicago there was made available for these experiments the temperature-humidity control equipment of its Botany Department. The writers wish also to express appreciation of the cooperation of C. S. CATHCART in making Kjeldahl determinations and of G. B. ULVIN'S assistance in making chlorophyll analyses.

surrounding air. Figure 1 shows that although a constant relative humidity may be maintained, the vapor pressure deficit of air varies as the temperature changes. It was found practically impossible, in the experiments cited, to maintain the same vapor pressure deficit at the extremes of temperature employed, but relatively easy to maintain the same relative humidity. The significance of vapor pressure deficit in relation to phases of metabolism other than transpiration is problematical. Because of this fact and because of the practical impossibility of maintaining the same vapor pressure deficit under these extremes of temperature, it was empirically chosen to maintain the same relative humidity at all temperatures. This

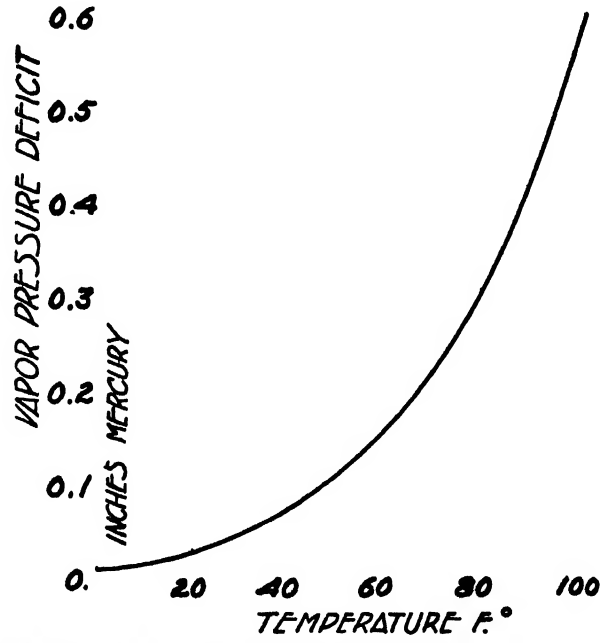


FIG. 1. Variation of vapor pressure deficit of atmosphere with rise of temperature at a maintained relative humidity of 70 per cent.

method of expression of the moisture content of the air is adhered to in this, as well as in the papers cited, and is commonly used in horticultural practice.

Tomato plants of the Bonny Best variety were employed for these experiments. They were grown for four weeks in sand culture with no external nitrogen supply, in new, washed, 10-inch clay pots, five plants to a pot, under prevailing greenhouse conditions. The pots were set in shallow enamelware pans which were kept constantly full of nutrient solution, which was applied daily in sufficient quantity completely to flush the sand.

On May 31, after the plants had been grown for four weeks with minus-N solution, some of them, later referred to as initial plants, were harvested for analysis and the remainder were placed in two glass inclosed chambers. The air in one chamber was maintained at 95 and in the other at 35 per cent. of saturation. The temperature was 70° F. (21° C.) in both cases. These conditions were constant day and night, with a variation in temperature of not more than $\pm 1°$ and in humidity of not more than ± 1.8 per cent. Sunlight and carbon dioxide supply were variable but the same for both humidity treatments. Condensation of moisture on the glass of the chambers did not occur as the surrounding air outside the chambers was not allowed to drop below 90° F.

Each chamber accommodated 20 pots, or 100 plants. After 12 hours under the humidity conditions indicated, half of the plants in each chamber received a complete or plus-NO_3 nutrient solution and the remainder continued to receive a solution lacking nitrogen. The nutrient solutions were applied daily, as already described, and the pans were kept constantly full of distilled water. In the low humidity chamber the surface sand at the top of the pots became dry to a depth of about 0.5 inch, but in the vicinity of the root systems the moisture was found to be in each case approximately 49 per cent. of the total water-holding capacity of the sand. With the same nutrient treatment, differences observed in growth and metabolism were brought about solely as the result of differences in humidity, although obviously all factors of environment influenced the response obtained.

Methods of chemical analysis of the tomato plants have already been described (**11**).

Results

Initial plants

The initial plants which were used for analysis and others of the same lot which were employed for experimental treatment were selected from a population of about 700, and were uniform in size, quality, and appearance. They were collected for analysis and experimental treatment begun on May 31, after the plants had been grown as already described with no external nitrogen supply. At that time they exhibited all the usual symptoms of nitrogen deficiency; and like the initial plants of the temperature experiments, may be described as follows: The stems were about 25 cm. long but stiff and woody. The base of the stem was purplish blue to light yellow. The upper two or three leaves were fairly dark green but the lower leaves were distinctly yellow with purple veins. There were practically no blossoms present. The root system was unusually large in proportion to the tops and consisted of many fine white extensive roots. The plants were high

in carbohydrates but very low in all forms of organic nitrogen, and contained neither nitrate nor ammonium (tables I–IV). Cell walls of mechanical and conductive tissue were thick, and starch was observed in large quantities in all parenchymatous tissue nearly to the tip of the stem.

Plants deficient in nitrate at 35 per cent. humidity

The plants deficient in nitrate were harvested for analysis on June 8, nine days after being continuously subjected to 35 per cent. humidity. During that period there was no noticeable change in appearance and but little change in chemical composition as compared with their initial condition on May 31 (tables I–IV).

Plants which received complete nutrient solution at 35 per cent. humidity

These plants absorbed nitrate instantly and in about four hours nitrate was present in abundance in all parts of the plant (**4**). Following absorption, assimilation of nitrate undoubtedly took place, for in nine days the stems increased nearly 100 per cent. in concentration of organic nitrogen (tables III, IV). The elaboration of nitrate necessarily involves utilization of carbohydrates, and as might be expected there occurred a decrease in sugars and starch (tables I, II).

Growth of these plants, however, was extremely slow for nitrate-supplied plants at 70° F. (**11**). The stems increased in length only 5–7 cm., and although there was some expansion of apical leaves they lacked vigor and succulence. They were comparatively thick, stiff to the touch, slightly mottled green in appearance, and even lower in chlorophyll (table V) than the leaf blades of the plants grown with no nitrate but with 95 per cent. humidity. It was further found that the stomatal apertures of the low humidity plants were small and that stomata were comparatively few in newly expanded leaves (**4**).

The plants exhibited no symptoms of wilting yet gave as a whole the general impression of dryness and stiffness. Microscopic observation (**4**) also showed that there was differentiation of heavy walled xylem and mechanical tissue, practically to the embryonic stem tip. In connection with this general lack of succulence, attention is called to the fact that although the plants were not low in total assimilated nitrogen, much of it was in the form of complex comparatively immobile proteins (tables III, IV).

Plants deficient in nitrate at 95 per cent. humidity

During the 9-day period at 95 per cent. humidity, these plants with no external nitrogen supply decreased materially in all determined forms of

TABLE I

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS–N		PLUS–NO_3	
		35	95	35	95
	%	%	%	%	%
Dry matter	14.80	15.20	13.80	11.20	10.40
Reducing sugars	8.60	6.30	5.70	4.98	2.98
Sucrose	3.51	4.45	1.05	3.75	2.05
Total sugars	12.11	10.75	6.75	8.73	5.03
Starch and dextrin	16.61	18.34	14.57	14.90	11.57
Total carbohydrates	28.72	29.09	21.32	23.63	16.60

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS–N		PLUS–NO_3	
		35	95	35	95
	%	%	%	%	%
Reducing sugars	1.27	0.96	0.79	0.56	0.31
Sucrose	0.52	0.67	0.14	0.42	0.21
Total sugars	1.79	1.63	0.93	0.98	0.52
Starch and dextrin	2.46	2.79	2.01	1.67	1.20
Total carbohydrates	4.25	4.42	2.94	2.65	1.72

carbohydrates, as shown in tables I and II. The percentage and quality of nitrogenous material in whole stems (tables III, IV) shows a slight increase in concentration of soluble organic nitrogen, and especially in percentage of the simpler, more mobile forms. Accompanying these changes the stems elongated from 3 to 4 cm. and lost much of the purplish blue of anthocyanin. The apical leaves also expanded, the plants became definitely darker green, and in the upper part more succulent. There was likewise found a remarkably high concentration of chlorophyll and increase in chloroplasts (**4**) in the leaf blades as compared with comparable plants lacking nitrate at 35 per cent. humidity (table V).

As might be anticipated, the stomatal apertures were found to be much more open at high than at low humidity, although they were not closed in any case (4).

Plants which received complete nutrient solution at 95 per cent. humidity

Absorption and translocation of nitrate, as nearly as could be determined by microscopic observation (4), apparently took place at about the same rate as at 35 per cent. humidity. On a percentage basis the amount of assimilated nitrogen would not appear to be greater at high than at low humidity (tables III, IV), but the plants at high humidity increased much

TABLE III

Nitrogenous fractions of whole stems expressed as percentage of dry matter

	Percentage humidity				
	May 31	June 8			
	Initial plants	Minus–N		Plus–NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.530	0.570	0.600	1.388	1.340
Protein N	0.418	0.478	0.480	0.788	0.420
Nitrate-free soluble N	0.112	0.092	0.120	0.600	0.920
Basic N	0.050	0.053	0.038	0.214	0.200
Amino N	0.043	0.029	0.060	0.258	0.494
Amide N	0.014	0.009	0.022	0.072	0.134
Other N	0.005	0.001	0.000	0.056	0.092
Nitrate N	None	None	None	0.466	0.500
Total N	0.530	0.570	0.600	1.854	1.840

more in volume than did those in the dry atmosphere, and, containing about the same percentage of total organic nitrogen on a green weight basis, must therefore have synthesized a considerably greater absolute amount. Necessarily accompanying comparatively rapid nitrate assimilation there occurred a marked decrease in carbohydrates (tables I, II).

Whereas the nitrate supplied plants at 35 per cent. humidity were woody and stiff, these were of extreme succulence. The newly expanded leaves had many stomata per unit area and the apertures were relatively large. The blades were thin, of an even dark green, and high in chlorophyll (table V). Not only were these organs thin and succulent, but there was only limited differentiation of xylem and mechanical elements in the stem tissue which developed during the 9-day period at high humidity. Further

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS-N		PLUS-NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.078	0.087	0.083	0.155	0.139
Protein N	0.062	0.073	0.066	0.088	0.044
Nitrate-free soluble N	0.016	0.014	0.017	0.067	0.095
Basic N	0.007	0.008	0.005	0.024	0.021
Amino N	0.006	0.004	0.009	0.029	0.051
Amide N	0.002	0.001	0.003	0.008	0.014
Other N	0.001	0.001	0.000	0.006	0.009
Nitrate N	None	None	None	0.052	0.052
Total N	0.078	0.087	0.083	0.207	0.191

cell division at the stem tip was rapid, as is apparent from the fact that the stems elongated from 12 to 15 cm.

Correlated with these growth responses it was found (tables III, IV) that an exceptionally high percentage of the total organic nitrogen was made up of amide and comparatively simple amino acids. This is in striking contrast to the quality of organic nitrogen found in the slow growing non-succulent plants at 35 per cent. humidity. These were notably high in complex proteins.

During the brief period of these experiments the roots were not noticeably affected by any of the experimental treatments.

TABLE V

CHLOROPHYLL IN THE LEAF BLADES OF TOMATO; JUNE 8

	PERCENTAGE HUMIDITY			
	MINUS-N		PLUS-NO_3	
	35	95	35	95
	%	%	%	%
Percentage of green matter	0.087	0.102	0.093	0.125
Relative values*	88	103	94	126

* Calculated using as 100 tomato plants which were subjected to plus-nitrate treatment at 70° F. and 85 per cent. humidity (11).

Discussion

Low humidity during a period of only eight days exerted a pronounced effect that was apparent in the external appearance of the tomato plants of these experiments, in that they lacked succulence, were low in chlorophyll (table V), and grew slowly as compared with comparable plants grown at high humidity. This response seems logical, however, as transpiration is greatly accelerated under conditions of low atmospheric moisture (**6, 10**). But such a marked external effect could scarcely take place were it not accompanied by internal changes. Chemical analyses (tables I–IV) show that internal changes did occur, and that they were of a type that usually (**8, 11, 12, 14, 16**) takes place in plants under conditions which promote carbohydrate accumulation.

The plants at 35 per cent. humidity with no external nitrogen supply, and therefore unmodified in carbohydrate metabolism by nitrate assimilation (**11**), were relatively high in dry matter and correspondingly low in moisture. They contained a high percentage of carbohydrates in the stems (tables I, II) and in all other plant parts. The nitrate supplied plants at low humidity, although lower in carbohydrates, owing to assimilation of nitrate (tables III, IV), were nevertheless much higher in concentration of sugars and starch than the plants with comparable nutrient treatment but at 95 per cent. humidity.

Further contributing to the high percentage of dry matter were the relatively thick cell walls of xylem and mechanical tissue found in the plants grown with low atmospheric moisture. This seems (**2, 9, 10**) to be a very common effect of low humidity. Yet it should also be emphasized that thick cell walls were developed under conditions of high humidity when nitrate was withheld, and there was thereby (**11**) maintained in the plant a fairly high concentration of carbohydrates (tables I, II). Cell wall thickness, with the possible exception of the calcium deficient plant (**17**), seems to be intimately associated with the carbohydrate content (**8, 13, 14, 15, 16, 23**). When carbohydrates are abundant cell walls are thick and the plant is stiff; when carbohydrates are low cell walls are thin and the plant is usually succulent. A wide variety of combinations of environmental conditions may modify the carbohydrate content of plants and therefore cell wall thickness.

Although the plants of these experiments were not grown to the fruiting stage, it may be mentioned that low humidity has been said (**2, 10**) to hasten flowering and fruiting. This also seems logical, as it has been demonstrated repeatedly (**8, 13, 14, 15**) that reproduction in plants takes place under conditions where carbohydrates are available in abundance rather than where carbohydrates are deficient. It may again be pointed out, how-

ever, that by withholding nitrate, carbohydrates were maintained in high concentration in the plants even at 95 per cent. humidity (tables I, II).

When grown with a complete nutrient solution, however, the plants subjected to high atmospheric moisture were low in carbohydrates (tables I, II), especially in the newly developed tissue. They therefore produced thin cell walls. Further, new growth was rapid and the plants were extremely succulent. Coupled with a comparatively low carbohydrate and dry matter content, high percentage of moisture, rapid growth, and succulence, there was found (tables III, IV) a very high concentration of the simpler soluble forms of organic nitrogen as amino, amide, and other nitrogen but the percentage of complex, relatively immobile proteins was low. This, however, is not a situation in any respect peculiar to high humidity. The less complex water soluble forms of organic nitrogen in the plant seem generally (**1, 12, 13, 14, 21**) to be present in abundance when carbohydrates are comparatively low and the moisture content is high.

At low humidity, however, under conditions of carbohydrate accumulation, slow growth, and lack of succulence, there was found (tables III, IV) in the nitrate supplied plants a fairly high concentration of total elaborated nitrogen. Yet much of it was in the form of complex, relatively immobile, dehydrated protein. An explanation is not attempted, but the general effect of dehydration apparently might be anticipated. It may be mentioned that EMIL FISCHER and others have employed dehydration in the synthesis of polypeptides from amino acids, and that storage proteins do not appear in seeds until desiccation begins (**3**). Condensation of simple amino acids to protein seems generally (**3, 12, 13, 14**) to occur with loss in moisture and increase in carbohydrates in the plant, and is not in any sense peculiarly a result of low humidity.

PART II. APPLE

The experimental technique employed and the results obtained were much alike for both tomato and apple. A comparatively brief presentation will suffice, therefore, to show that effects of relative humidity on apple are in harmony with the responses exhibited by tomato when subjected to similar conditions of humidity.

Experimental methods

The trees employed for this work were of the Rome variety. They had been propagated in the nursery as whole root grafts and were carefully selected 24-inch whips. On April 4, 1933, after several months in a cold storage cellar, 250 uniform trees were selected, washed free of adhering soil, the fine roots removed, and the dormant trees set in nitrogen-free quartz sand, in clean 3-gallon self-draining, glazed crocks, four trees per

crock. The tops of the trees were then pruned back to a uniform height of 25 cm. above the surface of the sand and all but the most distal bud was removed. This was done to eliminate variation in the number of shoots per tree, which would have made difficult the accurate comparisons of amount and quality of growth.

The crocks were placed in shallow enamelware pans and received daily applications of nutrient solution (**20**) in quantity sufficient to flush the sand. The pans were kept full at all times by the addition of distilled water. The water level in the pans did not extend more than 1 cm. above the bottom of the inside of the crocks. The sand adjacent to the roots was thus maintained at 52 per cent. of saturation.

On June 4, after the trees had been grown in sand for two months under usual greenhouse conditions, but with minus-N solution (**11, 20**), some of them, later referred to as *initial trees,* were harvested for analysis. At the time of this analysis and on all subsequent occasions, harvests were made shortly before sunrise. The remainder of the trees were placed in the humidity chambers. The air in one chamber was maintained at 40 and in the other at 95 per cent. relative humidity, with a temperature of 75° F. in both cases.

The plant population and other conditions were essentially the same as for tomato. After 12 hours in the chambers, nitrate was applied to half the plants of each group. The methods of chemical analysis of the apple trees have already been described (**18, 20**). The *current stem* and *leaves* were the only plant parts which were analyzed macrochemically. In other portions of the trees there was little noticeable difference between comparable nutrient series during the 18-day period of these experiments.

Results

Initial trees

Although the initial trees had no external nitrogen supply when they were shifted to the humidity chambers on June 4, they were nevertheless all actively growing with no sign of terminal bud development. The current stems were about 60 cm. in length and rather dark red, except the upper third of the stem which was comparatively soft and green in color. A few of the lower leaves were slightly yellowish green although the foliage of the remainder of the stem was dark green. White fibrous roots were very abundant and extensive, but of comparatively small diameter. This is a condition frequently exhibited by apple trees and other plants if grown with a limited nitrogen supply and opportunity for carbohydrate accumulation. Starch was observed in abundance nearly to the tips of the finest roots. Nitrate was not present as there was none in the nutrient medium.

TABLE VI

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Dry matter	27.00	39.50	34.50	33.00	29.00
Reducing sugars	5.83	4.27	3.15	3.22	1.70
Sucrose	1.29	2.41	1.20	1.50	0.82
Total sugars	7.12	6.68	4.35	4.72	2.52
Starch and dextrin	4.13	7.72	6.55	4.26	3.00
Total carbohydrates	11.25	14.40	10.90	8.98	5.52
CURRENT STEM					
Dry matter	26.50	39.00	34.50	33.50	29.50
Reducing sugars	1.90	2.10	1.90	1.00	0.78
Sucrose	0.85	1.00	0.55	0.27	0.11
Total sugars	2.75	3.10	2.45	1.27	0.89
Starch and dextrin	6.20	5.90	3.97	3.52	1.59
Total carbohydrates	8.95	9.00	6.42	4.79	2.48

TABLE VII

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Reducing sugars	1.57	1.69	1.09	1.06	0.49
Sucrose	0.35	0.95	0.41	0.49	0.24
Total sugars	1.92	2.64	1.50	1.55	0.73
Starch and dextrin	1.11	3.05	2.26	1.41	0.87
Total carbohydrates	3.03	5.69	3.76	2.96	1.60
CURRENT STEM					
Reducing sugars	0.50	0.82	0.66	0.34	0.23
Sucrose	0.23	0.39	0.19	0.09	0.03
Total sugars	0.73	1.21	0.85	0.43	0.26
Starch and dextrin	1.64	2.30	1.37	1.18	0.47
Total carbohydrates	2.37	3.51	2.22	1.61	0.73

Associated with these growth responses and those exhibited by the aerial organs, there was found in the leaves and current stem an abundance of sugars and starch (tables VI and VII) and a moderate concentration of organic nitrogen but no nitrate (tables VIII and IX).

TABLE VIII

NITROGENOUS FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Total nitrate-free N	2.73	2.42	2.56	3.10	3.40
Protein N	2.29	2.17	2.26	2.68	2.82
Nitrate-free soluble N	0.44	0.25	0.30	0.42	0.58
Nitrate N	None	None	None	None	None
CURRENT STEM					
Total nitrate-free N	1.39	0.78	0.94	1.05	1.42
Protein N	0.57	0.48	0.50	0.58	0.65
Nitrate-free soluble N	0.82	0.30	0.44	0.47	0.77
Nitrate N	None	None	None	None	None

TABLE IX

NITROGENOUS FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS–N		PLUS–NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Total nitrate-free N	0.73	0.96	0.88	1.02	0.99
Protein N	0.62	0.86	0.78	0.88	0.82
Nitrate-free soluble N	0.11	0.10	0.10	0.14	0.17
Nitrate N	None	None	None	None	None
CURRENT STEM					
Total nitrate-free N	0.37	0.30	0.32	0.35	0.42
Protein N	0.15	0.19	0.17	0.19	0.19
Nitrate-free soluble N	0.22	0.11	0.15	0.16	0.23
Nitrate N	None	None	None	None	None

Trees deficient in nitrate at 40 per cent. humidity

The trees, under these conditions of low humidity with no external nitrogen supply, apparently ceased terminal growth almost at once. At least the current stems made no measurable increase in length, and in 5 or 6 days it was clearly evident that all the trees of this group were forming a terminal bud. Simultaneously the foliage became yellowish green and the tip of the stem much stiffer and darker red. Internally this was associated with heavy deposition of starch and thickening of cell walls, especially near the tip of the stem. At the end of 18 days the leaves and current stem had increased greatly in percentage of dry matter and carbohydrates, as shown in tables VI and VII. On a dry weight basis there occurred a decrease in concentration of nitrogen (table VIII), although there was apparently translocation of soluble organic nitrogen from the current stem to the leaves where there was seemingly condensation to proteins, as indicated in table IX. As already pointed out in case of tomato, this type of response seems frequently to occur with loss of moisture and increase in dry matter in the plant (**3, 11, 12, 13, 14, 21**).

Trees which received complete nutrient solution at 40 per cent. humidity

The trees at low humidity which were supplied with nitrate absorbed it instantaneously, and nitrate as usual was observed only in the fibrous roots. That the trees assimilated nitrate was evident by the appearance of nitrite and a noticeable decrease in starch in the fibrous roots only 20 hours after nitrate was absorbed. Two days after the complete nutrient solution was applied the foliage became darker green, although dull green, and the current stem and petioles gradually decreased in intensity of red color. During the 18-day period the trees increased 5 to 8 cm. in length of current stem, and materially in area of the leaves (near the stem tip) which were only partially expanded when the experiments were commenced. At the end of 18 days, however, all of the trees had terminal buds and shortly before that time the stem tips were rapidly becoming woody and darker red. It may be pointed out that development of terminal buds was therefore much slower than in case of the trees lacking nitrate at low humidity, also that the nitrate supplied trees were much lower in dry matter and in carbohydrates (tables VI and VII). Maturity of vegetative or reproductive organs is frequently associated with accumulation of carbohydrates.

At the time of harvesting, dry matter in the current stem was considerably higher in the trees which received nitrate under the conditions of low humidity, than in the initial trees (table VI). This would seem to be due, at least in part, to the marked increase in thickness of cell walls which

occurred especially in the upper third of the current stem. Accompanying increase in thickness of cell walls, there was a decrease in percentage of the determined carbohydrate fractions (tables VI and VII). Polysaccharides other than starch may very well have increased, however, as is indicated by the work of MURNEEK (**10a**).

In the same trees the increase in percentage dry matter in the leaves (table VI) may have been due partly to relatively rapid loss of water through transpiration under the conditions of low atmospheric moisture. There was, however, no noticeable sign of wilting of these trees nor of any of the others of these experiments. On the other hand, the cuticle of the leaves of both series at 40 per cent. humidity was comparatively thick as were also the cell walls of veins and mesophyll.

Cell walls are made up partly of cellulose and other carbohydrate materials which are derived presumably through condensation of the simpler carbohydrates, resulting in a decrease of those in storage unless supplied through new synthesis. It should also be pointed out that although nitrate assimilation is limited mainly to the fibrous roots in apple (**18, 19**), the aerial organs must eventually supply the carbohydrates utilized in the synthesis of proteins from nitrate. Therefore both of these factors, assimilation of nitrate and thickening of cell walls, would presumably involve utilization of carbohydrate fractions such as those determined (tables VI and VII). This increase of dry matter with decrease in percentage of determined carbohydrates is in contrast to the occurrence in an herbaceous plant such as tomato, where fluctuations in percentage dry matter commonly correspond with changes in concentration of the simpler metabolic carbohydrates such as sugars and starch.

The concentration of organic nitrogen on a dry weight basis (table VIII) is higher in the current stem and leaves of the trees supplied with nitrate at 40 per cent. humidity than in the trees lacking an external nitrogen supply under the same conditions of atmospheric moisture. Expressed as percentage of green weight matter, there is very little difference between the two groups of trees (table IX). It should be recalled, however, that the trees which received nitrate increased considerably more in volume than those lacking it. In both cases, accompanying the conditions of low humidity and associated with the development of terminal buds there was a marked decrease in soluble organic nitrogen and increase in the relatively complex protein fraction (table IX). A similar response of the tomato plants is described earlier in this paper.

Both groups of trees at 40 per cent. humidity exhibited stomata which were approximately closed day and night. The leaves near the tip of the stem which made all or most of their growth during the 18-day experimental period were relatively small in area when fully developed. Yet

when completely expanded these leaves had a smaller number of stomata per unit area than comparable leaves at high humidity. The palisade and spongy mesophyll of the former were also very compact, with comparatively small cells and small intercellular spaces, and had, as already mentioned, thick cell walls and cuticle.

Trees deficient in nitrate at 95 per cent. humidity

The trees under high humidity conditions with no external nitrogen supply gradually increased in rate of growth of the current stem. The foliage became darker green and there was considerable increase in number and area of leaves. When the trees were harvested for analysis on June 22, the current stems had increased in length from 8 to 12 cm. In no case was there any sign of a terminal bud. The new stem growth was of comparatively small diameter, and in the upper third of the stem it was considerably less woody than in the case of either group of trees at 40 per cent. humidity. It will also be recalled that during the same experimental period, terminal buds appeared on all the trees of both nutrient series at low humidity.

Accompanying the conditions of high atmospheric moisture and associated with continuous linear stem growth and development of new leaves, there was found in the current stem and leaves of the trees without nitrate a very much lower percentage of dry matter and carbohydrates than in the trees lacking nitrate at low humidity (tables VI and VII). The contrast in the upper third of the current stem, however, was much more striking than is indicated by the macroanalysis of whole stems. In the stem tip there was comparatively little starch and the cell walls were relatively thin. In the lower portion of the stem there was much starch, and considerable increase in thickness of cell walls had occurred since the initial observations were made on June 4. This would apparently account, at least in part, for the increase in percentage dry matter as compared with that found in the current stem of the initial trees (table VI).

The leaves of the trees which were subjected to high humidity with no external nitrogen supply present a situation that requires explanation. The analytical sample *leaves* consisted of young and old leaves. The latter contained considerable starch. In fact, separate analysis of a group of lower leaves which turned yellow and abscissed before the end of the experimental period showed them to be high in starch but extremely low in nitrogen. It may be remarked that this was the only series which exhibited abscission or severe yellowing of leaves. It would seem that the low concentration of nitrogen in these lower leaves may have been associated with early senescence. The low concentration of nitrogen may also have limited, directly or indirectly, diastatic digestion in those older leaves which did

not abscise. There would thus be maintained in the leaf sample as a whole a comparatively high percentage of dry matter and carbohydrates (tables VI and VII).

The leaves of the upper third of the stem were dark green and extremely low in starch. The young leaves (near the tip of the stem) which expanded during the period at high humidity were of relatively large area but not so thick as comparable leaves under conditions of low humidity. The cuticle and cell walls were also thinner, and the palisade and spongy mesophyll very much less compact than in case of the leaves which developed under the conditions of low atmospheric moisture. As might be anticipated, the stomata of the trees of both nutrient series were wide open during the day at 95 per cent. humidity, although closed at night.

It may again be emphasized that the trees with no external nitrogen supply stopped linear stem growth almost immediately when shifted to low humidity, and rapidly developed a terminal bud. In striking contrast, accompanying high humidity there occurred the considerable increase in volume of tops and decrease in carbohydrates in the upper leaves and current stem that has already been described, even though there was no nitrogen in the nutrient medium. Between the roots of these two groups of trees there was little, if any, difference in appearance and chemical composition.

It has already been mentioned that there was loss of nitrogen from the older leaves of the trees grown without nitrate at 95 per cent. humidity. It is also apparent that there was a decrease in soluble organic nitrogen in the current stem as shown in tables VIII and IX. Obviously the considerable increase in volume of the trees could not have occurred without a drop in concentration of total nitrogen. This is clearly indicated in table IX. It would seem that in these apple trees, as in tomato, the condition of high humidity directly or indirectly resulted in a decrease in percentage of carbohydrates, accompanying which there occurred proteolysis and increased growth as already recorded. As discussed in greater detail in Part I, hydrolysis of proteins seems frequently to follow or accompany decrease in dry matter in the plant (**5, 11, 12, 13, 14, 21**).

Trees which received complete nutrient solution at 95 per cent. humidity

The trees which received nitrate at 95 per cent. humidity absorbed it instantly and it was observed only in the fine roots. Reduction to nitrite occurred in these organs, and other observations concerning the fibrous roots were essentially the same as recorded for the trees which were supplied with the complete nutrient solution at low humidity.

The leaves became noticeably darker green about 20 hours after ap-

plication of nitrate, and at the end of the 18-day period of these experiments the foliage of even the lower leaves was almost black-green. During this interval the current stems increased 12 to 16 cm. in length. There was no sign of a terminal bud and almost all red pigmentation disappeared from the current stem, which was very much softer near the tip than in case of any of the other series.

Associated with these responses there was found to be very little starch in the upper third of the stem and there the cell walls were comparatively thin. The remainder of the current stem exhibited considerable cell wall thickening and some starch, but was very much lower in starch and had thinner cell walls than comparable portions of the trees of the other groups. The relatively low percentage of carbohydrates in the current stem and leaves is clearly indicated in tables VI and VII. In general, the lower leaves contained more starch than the tip leaves but neither was high in starch.

In structure the newly expanded but fully developed leaves were similar to comparable leaves of the trees grown without nitrate under the same conditions of high humidity. The former, however, were considerably larger in area and relatively thinner. Cell walls and cuticle were very thin and the palisade and spongy mesophyll were loosely constructed of large cells and large intercellular spaces. Stomata were wide open during the day but apparently closed at night.

Associated with relatively rapid growth, nitrate assimilation, and decrease in carbohydrates, there was a much higher percentage of soluble organic nitrogen in the current stem and leaves than in case of any of the other groups of trees harvested on June 22 (tables VIII and IX). A similar situation has frequently been observed and is discussed more fully in connection with the tomato. They apparently responded in an analogous manner. Although the trees of this group increased rapidly in volume there was maintained in the current stem and leaves a high percentage of total organic nitrogen (tables VIII and IX). Presumably there was therefore a considerable increase in absolute amount of organic nitrogen newly synthesized from nitrate.

Summary

Tomato

1. Tomato plants were grown in sand maintained at 49 per cent. of saturation in all cases. The experiments were carried on in glass inclosed chambers at a constant temperature of 70° F. (21° C.). Sunlight and carbon dioxide supply were variable but at any given time were the same for each humidity treatment. One group of plants was grown at 35 and the other at 95 per cent. humidity.

2. The plants grown with a complete nutrient solution at 35 per cent. humidity were lighter green and contained less chlorophyll than those subjected to high atmospheric moisture. They grew slowly, had relatively thick leaves, stiff stems, and in general lacked succulence. Carbohydrates were relatively high and cell walls were thick. There was a fairly high concentration of total organic nitrogen but much of it was as complex insoluble protein.

3. Comparable plants at 95 per cent. humidity were dark green and high in chlorophyll. They grew rapidly, had relatively thin leaves, and the leaf and stem tissue which developed during the period of high atmospheric moisture was comparatively thin walled and succulent. Carbohydrates were relatively low and much of the organic nitrogen was water soluble.

4. It is pointed out and shown experimentally that these responses are typical of, but not peculiar to, the respective humidity treatments.

Apple

1. Young Rome apple trees were grown in sand culture under conditions similar to those described for tomato.

2. All the trees at 40 per cent. humidity had terminal buds at the end of 18 days, although those receiving the complete nutrient solution formed them much more slowly than the trees lacking nitrate under the same conditions. Accompanying low humidity the foliage became lighter green and the current stems darker red. Associated with these responses there tended to be carbohydrate accumulation and apparently condensation of the simpler forms of organic nitrogen to complex proteins.

3. Comparable trees at 95 per cent. humidity had darker green leaves which were relatively thin. The current stems exhibited little red color and elongated rapidly even in case of the trees lacking nitrate. During the 18-day period of these experiments the current stem tips remained comparatively soft and none of the trees formed terminal buds. Correlated with these responses carbohydrates were found in relatively low concentration, there was less indication of protein condensation, and cell walls were comparatively thin.

New Jersey Agricultural Experiment Station, and
University of Chicago

LITERATURE CITED

1. Chibnall, A. C., and Miller, E. J. Some observations on the distribution of nitrogen in plant extracts that contain a high proportion of nitrate nitrogen. Jour. Biol. Chem. **90**: 189–196. 1931.
2. Eberhardt, Ph. Influence de l'air sec et de l'air humide sur la forme

et sur la structure des végétaux. Ann. Sci. Nat. Bot. VIII. **18**: 61–152. 1903.

3. ECKERSON, SOPHIA H. Microchemical studies in the progressive development of the wheat plant. Washington Agr. Exp. Sta. Bull. 139. 1917.
4. ————. Effect of temperature and humidity upon nitrate reducing ability of the tomato. Contributions from the Boyce Thompson Inst. (Unpublished).
5. FAGAN, T. W. The variation in the moisture and nitrogen content of the potato during growth and storage. Welsh Jour. Agr. **1**: 110–113. 1925.
6. KIESSELBACH, T. A. Transpiration as a factor in crop production. Nebraska Agr. Exp. Sta. Res. Bull. 6. 1916.
7. KOSTYCHEV, S. Plant respiration. Blakiston. Philadelphia. 1927.
8. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
9. LEBEDINCEV, E. Physiologische und anatomische Besonderheiten der in trockener und feuchter Luft. gezogenen Pflanzen. Ber. deutsch. bot. Ges. **45**: 83–96. 1927.
10. MAXIMOV, N. A. The plant in relation to water. Allen and Unwin. London. 1929.

10a. MURNEEK, A. E. Hemicellulose as a storage carbohydrate in woody plants, with special reference to the apple. Plant Physiol. **4**: 251–264. 1929.

11. NIGHTINGALE, G. T. Effects of temperature on metabolism in tomato. Bot. Gaz. **95**: 35–58. 1933.
12. ————, and ROBBINS, W. R. Some phases of nitrogen metabolism in polyanthus narcissus (*Narcissus tazetta*). New Jersey Agr. Exp. Sta. Bull. 472. 1928.
13. ————, and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
14. ————, ————, and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
15. ————, ————, and ————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.

16. ————, Addoms, Ruth M., and Blake, M. A. Development and ripening of peaches as correlated with physical characteristics, chemical composition, and histological structure of the fruit flesh. III. Macrochemistry. New Jersey Agr. Exp. Sta. Bull. 494. 1930.
17. ————, ————, Robbins, W. R., and Schermerhorn, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
18. ————. Ammonium and nitrate nutrition of dormant Delicious apple trees at 48° F. Bot. Gaz. **96**: 437–452. 1934.
19. ————, and Blake, M. A. Effects of temperature on the growth and composition of Stayman and Baldwin apple trees. New Jersey Agr. Exp. Sta. Bull. (Unpublished.)
20. ————, and ————. Effects of temperature on the growth and composition of Elberta peach trees with notes on the responses of other varieties. New Jersey Agr. Exp. Sta. Bull. (Unpublished.)
21. Pearsall, W. H., and Ewing, J. The relation of nitrogen metabolism to plant succulence. Ann. Bot. **43**: 27–34. 1929.
22. Stiles, W. Photosynthesis. Longmans Green. London. 1925.
23. Welton, F. A. Lodging in oats and wheat. Bot. Gaz. **85**: 121–151. 1928.

THE SOIL AS DIRECT SOURCE OF CARBON DIOXIDE FOR ORDINARY PLANTS[1]

BURTON E. LIVINGSTON AND RUTH BEALL

Introduction

About the middle of the nineteenth century, when the photosynthetic process in chlorophyll-bearing cells was becoming appreciated, the question naturally arose as to whether the CO_2 necessary for that process in ordinary plants might be in part derived from the soil as well as from the free air. It was soon shown that the free air is generally the main source of the CO_2 of photosynthesis in green plants with aerial exposure, and that conclusion has remained well supported up to the present time; there is now no reason to question its correctness. But some experiments were reported which indicated that a portion of the CO_2 decomposed in green leaves had been derived not from the free air about those organs but from other parts of the plant body and even from the soil. Many recent writers appear to have ignored the results of those experiments, as well as the basic physical principles involved, and it is probably safe to suppose that the free air is now generally regarded as the *only* source of CO_2 for ordinary plants. That only a relatively small portion of the CO_2 actually absorbed can be supposed to enter the plant through the roots ought not to lead to the complete neglect of root absorption of CO_2, especially when it is recalled that the soil solution has generally a considerable concentration of carbonate ion, that large amounts of water are absorbed through root systems, and that almost all of the absorbed water is regularly given off in transpiration through green tissues. We submit a brief summary of the status of this fundamental question, together with some discussion of the logical possibilities and an account of a few experiments.

To conserve space we shall refer to the carbonate ions (HCO_3^- $CO_3^=$) in aqueous solution as *dissolved* CO_2. Since gaseous CO_2 cannot penetrate to active chloroplasts (which are surrounded by and thoroughly impregnated with water), it is safe to suppose that all CO_2 decomposed in green cells must be in watery solution before its reduction may occur. It is not necessary for our present purpose to attempt to distinguish between the hypothetical carbonic acid (H_2CO_3), the carbonates, and the carbonate ions, nor is it necessary to dwell on the consideration that a very small portion of

[1] Botanical contribution from the Johns Hopkins University, no. 124. A brief abstract of portions of the material published here was presented by LIVINGSTON and BEALL at the Des Moines meeting of the American Society of Plant Physiologists. December, 1930.

the CO_2 of the plant body is surely dissolved in solvents other than water, such as oils and waxes.

We shall consider just those autotrophic plant forms that have root systems in soil or in aqueous solution while stems and leaves are exposed to the free air. Completely submerged aquatics that absorb CO_2 surely receive it exclusively from the surrounding solution,—the water of lakes, streams, the sea,—and direct absorption from the free air is naturally out of the question for them.

BOUSSINGAULT (7), in 1844, showed that most if not all of the carbon of the ordinary green plant is derived from the free air, but UNGER (40) thought that absorption from the free air would not account for all the carbon in the plant.

CORENWINDER (13) inclosed a part of a cut branch in a glass container with air from which CO_2 had been removed, while the remainder of the branch was in the open air. Development was more pronounced in the container. He attributed the difference to more favorable temperature in the container, and concluded that the inclosed leaves had received their CO_2 from the uninclosed part of the branch. In support of the view that CO_2 may be derived from sources other than the free air, this writer mentioned an experiment of DE SAUSSURE (35), in which a leafy branch, still attached to a tree, was inclosed in a glass receiver with CO_2-free air; at the end of the experiment the oxygen content of the air in the receiver was found to have been notably increased. Obviously, however, this result might have been brought about without photosynthesis in the inclosed branch, if we suppose simply that some oxygen had found its way from other branches to the inclosed one and had there passed into the air space of the receiver.

BIRNER and LUCANUS, in 1866 (5), found that the first effect of an introduction of CO_2 into the nutrient solution in which oats were growing was a noticeable injury to the plants, but on continued addition of CO_2 those plants showed better development and higher dry weights than similar plants grown in the same nutrient solution but without added CO_2. They concluded that an extra supply of CO_2 in the solution had a favorable influence on the production of organic material, but they considered it an open question whether the additional CO_2 was absorbed by the roots and transported to the green parts or diffused from the solution into the air and absorbed therefrom by the leaves. Long afterwards FREE (18) reported that a bubbling stream of CO_2 passing through nutrient solution in which buckwheat plants were growing produced injury and death. Partial recovery ensued if, after the first day, the stream of CO_2 was replaced by a stream of air, but these plants remained smaller than the controls. Poisoning may have been due to CO_2 or possibly to some other cause.

BÖHM (6) grew plants of scarlet-runner bean, some in sand and some

in humus soil, each group being separately inclosed in a glass jar in which was air kept free from CO_2 by the presence of an open vessel of KOH solution. Finding no more starch in the plants grown in humus soil than in the others, he concluded that the extra supply of CO_2 presumably present in the humus soil could not be drawn upon.

In 1877 MOLL (**26, 27**), working in SACHS's laboratory, carried out some experiments similar to those of BÖHM, but more elaborate, and he also reached the conclusion that all CO_2 decomposed in leaves must come from the surrounding air, not from other parts of the plant. In an adequately illuminated, healthy green leaf, still attached to its plant and surrounded by CO_2-free air in a closed container, there was no evidence of starch formation even though other parts of the same plant were exposed to air unusually rich in CO_2; nor was starch formation apparently accelerated when the inclosed leaf was in ordinary air, which must have furnished some CO_2 directly. In these experiments of BÖHM and MOLL the rate of transpiration from inclosed leaves must have been very slow.

In 1878 VINES (**42**) reported that *Ricinus,* pumpkin, and maize plants, grown in closed chambers and without CO_2 in the air surrounding their leaves, gained in size less rapidly and showed much less starch than those grown in ordinary air. Although some starch was found in stomatal guard cells and in bundle sheaths of leaves grown without an aerial supply of CO_2, VINES decided that no CO_2 could have reached these leaves from other parts of the plant. In these experiments also, water loss by transpiration must have been very slow, although the CO_2-free air of the container was renewed each morning and two or three times throughout the day. In 1886 VINES wrote (**42**, pp. 82, 83), "There is no experimental evidence which would tend to shew that this property [of absorbing CO_2] is under any circumstances possessed by plants or parts of plants which do not contain chlorophyll." . . . "Recent investigations have, however, shewn that this theory [that a portion of the CO_2 required by a plant may be absorbed by the roots from the soil] is quite untenable."

SACHS, in whose laboratory the experiments of MOLL and VINES had been performed, may have been influential in leading these experimenters to such extreme conclusions, for in 1882 he wrote (**34**, p. 352), ". . . es eine durchaus unrichtige Ansicht ist, zu glauben, dass die in der Erde gewöhnlich reichlich enthaltene Kohlensäure von der Wurzeln aufgesogen und den Blättern zugeführt werde." He also wrote (**34**, p. 374) of MOLL's logen bis auf die neueste Zeit gehegt Ansicht, als ob die Kohlensäure von experiments, "Diese Versuche beweisen, dass die von den älteren Physioden Wurzeln aus in die Blätter geführt dort assimilirt würde, durchaus ungerechtfertigt ist." SACHS may have been intent on just putting an end to the old humus theory, that all of the carbon in ordinary green plants

was derived from the soil in the form of carbon compounds; it is difficult to imagine that he failed to realize that *some* CO_2 (be it ever so little) might be conveyed from roots to leaves under suitable sets of conditions. At any rate, the experimentation with which SACHS was acquainted furnished no basis for such sweeping statements as those just quoted, nor can such statements be justified by any subsequent experiments.

A year before the publication of these sentences by SACHS, PFEFFER (**30**, p. 200) had written with characteristic carefulness, " . . . dass eine Landpflanze fast die ganze Kohlensäure aus der die assimilirenden Organe umgebenden Luft schöpft, durch die Wurzeln und überhaupt die im Boden befindlichen Theile aber den Blättern nur wenig Kohlensäure zugeführt wird," . . . "so können doch kleine Mengen Kohlensäure den assimilirenden Organen von den Wurzeln aus zugeleitet werden." . . . "Uebrigens muss die Ausgiebigkeit der Transpiration, resp. die hierdurch veranlasste Wasser- und Gasbewegung in der Pflanze von Bedeutung sein für das Quantum Kohlensäure, welches vom Boden aus in die Pflanze gelangt." It is important that PFEFFER recognized that rapid transpiration might be expected to accelerate movement of CO_2 from roots to leaves.

Like PFEFFER, EWART (**17**, p. 569) recognized the possibility that CO_2, evolved within the plant or absorbed by its roots, might reach the green tissues by the internal route and might be reduced in photosynthesis. And in his translation of PFEFFER's Pflanzenphysiologie, he introduced no editorial modification of the German statement. The EWART translation (**31**, p. 329) states: ". . . a leaf or green stem absorbs directly from the atmosphere almost the whole of the carbon dioxide which it assimilates" . . . "Minute quantities of dissolved gas [CO_2] may . . . reach the leaves from the roots. . . . Indeed, the amount derived from the roots may, when transpiration is active, be sufficient to prevent the chloroplastids in the more deeply situated tissues at the base of a green stem from losing the power of assimilating carbon dioxide when exposed to light for prolonged periods in an atmosphere free from this gas."

MACDOUGAL (**20**) found that plants differed in their reaction to an atmosphere devoid of CO_2, but concluded that such growth as was possible under these conditions was due to food already present in the plant, since (**20**, p. 541) "the growth of a plant in an atmosphere free from CO_2 practically suspends the food-forming processes." The apparatus he used was similar to that of MOLL (**26**, **27**), discussed later in this paper, and there was no considerable transpiration.

No further experimentation on this question seems to have been reported until ZIJLSTRA's paper appeared in 1909 (**45**). Repeating one of MOLL's experiments, ZIJLSTRA used a number of monocotyledonous and dicotyledonous plants, including such forms as *Dahlia, Polygonum, Aesculus,*

Acer, Acorus, Zea, Triticum, Tradescantia. He exposed the tip portion of a leaf in an atmosphere free from CO_2 and supplied an abundance of this gas to another region of the same leaf. After a period of illumination of both regions starch appeared in the region that could not have received any CO_2 from the outside, indicating some movement of CO_2 through the tissue, but the distance traversed was apparently never more than a few centimeters. It was greater when the intercellular spaces of the intervening tissue were large, and conversely. In discussing ZIJLSTRA'S results, MOLL (**28**) admitted the inaccuracy of his own earlier conclusions as to the *absolute impossibility* of starch formation in one part of a leaf at the expense of CO_2 derived from an adjacent part, but he maintained (**28**, p. 668), "ZIJLSTRA'S results are, however, in complete agreement with the main result, formerly obtained by the speaker, according to which the carbon dioxide of the soil, even if it should be absorbed by the roots, cannot appreciably contribute to the synthesis of organic matter in the leaf." In ZIJLSTRA'S experiments, as in earlier ones, there was no considerable transpiration current, which might serve to accelerate movement of CO_2 from the region with external supply to the region without any such supply; what movement did occur was probably due wholly or almost wholly to simple diffusion through the tissues,—apparently largely to gas diffusion through intercellular spaces of the leaf.

MITSCHERLICH (**25**) grew oat plants in soil watered with tap water saturated with CO_2 and found no better yield from these plants than from similar ones watered with ordinary tap water. He conceived that any increased growth that might result from adding CO_2 to the soil might be due to increased solubility of soil materials, and concluded that, since his soil already possessed an abundance of CO_2, further addition did not improve growth.

In POLLACCI'S review (**32**) of earlier papers on this question, he pointed out some probable sources of error that might have led to the opinion that CO_2 is not absorbed at all by plant roots. He added a report on some new experiments, which may be summarized as follows: Maize seeds were allowed to germinate in an atmosphere free from CO_2 and the resulting plantlets were grown with their roots in nutrient solution to which CO_2 was added, while their leaves were in CO_2-free air. The plants that developed had dry weights greater than was shown for the seeds before they germinated, and POLLACCI stated that this could not have been true except by assimilation of CO_2 derived from the nutrient solution through the roots. In another experiment maple seedlings were first kept in darkness, with roots in nutrient solution to which CO_2 was added and with leaves in CO_2-free air, until sample leaves showed no trace of starch. Then these plants were exposed to natural light conditions. They developed well for several

months, and microscopic examination showed the presence of some starch in their leaves. Also, small plants of oak, maple, and horse-chestnut, rooted in humus soil and with their aerial organs in CO_2-free air, grew for several months and produced some starch in their leaves. From these experiments POLLACCI concluded that CO_2 absorbed by the roots of green plants may be the source of some carbon for starch formation in the green tissues.

In reporting a study of respiration of detached roots, CERIGHELLI (**11**, **12**) remarked that when a root is attached to the plant gaseous exchanges with the surrounding atmosphere are masked by phenomena connected with gaseous circulation within the plant; that water absorbed by roots carries CO_2 formed there to the upper parts of the plant where it becomes reduced by photosynthesis, and gases formed by respiration and photosynthesis in the aerial parts may be transferred to the roots. In his earlier paper (**11**) he stated that he had never verified CO_2 absorption by roots and thought, —contrary to POLLACCI, as he says,—that CO_2 originating in the root may play a more important rôle than is played by CO_2 derived from soil water.

In BREAZEALE'S discussion of carbon absorption by roots (**8**, p. 305) he wrote, "There are strong indications that the plant absorbs the CO_3 ion from solution by means of its roots." Nevertheless, in some experiments planned to throw light on the question as to whether carbon thus absorbed might eventually take part in the formation of carbohydrate or other complex organic compounds, he found that wheat plants grown under bell jars gave much smaller dry yields than were given by similar plants grown in the open, and he concluded (**8**, p. 307): "It seems quite evident that the plant is unable to substitute, to any appreciable extent, the carbon of the CO_3 radical absorbed by the roots for the carbon of the carbon dioxide taken in by the leaves." The reader needs to bear in mind that, despite its too general wording, this conclusion obviously refers just to the plants of the particular experiments in question; the evidence being insufficient to warrant its application to green plants in general. Furthermore, the environment of the plants under bell jars differed from the environment of those grown in the open in several ways, aside from the aerial supply of CO_2: the two environmental complexes were surely not alike with respect to illumination and to radiation in general; they presumably differed somewhat with respect to air temperature; and, above all, they must have differed greatly with respect to conditions that influence transpiration. Everything considered, these experiments appear to have no direct bearing on the broad question before us, but BREAZEALE was surely logically right in his first supposition, that at least some CO_2 must generally be absorbed by the roots of ordinary green plants.

BAMAL (**2**) considered CO_2 as a "fundamental fertilizer," noting some benefits that accrue to the soil from its presence therein. It is not clear,

however, whether he thought considerable amounts of CO_2 might reach the foliage *via* root absorption and internal transport, or regarded CO_2 from the soil as reaching the leaves mainly or wholly by passing first into the air.

According to his monograph on photosynthesis of carbon compounds in plants, apparently STILES was convinced that the free air is the only possible source of CO_2 for the kinds of plants we are considering, as is indicated or at least implied by such expressions as the following (**37**, pp. 3, 40): ". . . but it was not at first realized that it [the free air] is the only source of the carbon compounds of the plant . . .;" "When, however, it was realized that all the organic material in the plant is derived from the carbon absorbed by the leaf . . .;" "the absorption of carbon by the plant takes place exclusively by the intake of carbon dioxide by the leaves and other green organs of plants." On the other hand, SPOEHR did not fail to take what must be regarded as the logically correct view in this connection, for he wrote (**36**, p. 79), "It is conceivable, however, that conditions may exist in which the carbon dioxide absorbed by the roots may contribute to the total amount of carbon dioxide reduced by the plant."

STOKLASA (**38, 39**) took the definite position that considerable amounts of the CO_2 decomposed in the leaves of ordinary plants may reach the chlorophyll-bearing tissues *via* roots and stem, having been absorbed from the soil across the generally permeable boundary of the root system; and that the supply of this substance to the green tissues may in this way sometimes be considerably greater than might be accounted for by direct absorption across aerially exposed surfaces. He held that soil bacteria play an important rôle in accelerating CO_2 production in the soil, adding CO_2 to the atmosphere about the leaves as well as providing carbonate ions in the soil solution. He wrote (**38**, p. 5): "Diese Bikarbonate werden vom Wurzelsystem der Pflanze resorbiert und den Chlorophyllapparaten zugeführt. Den Pflanzen wird auf diese Weise eine grössere Menge Kohlenstoff in Form von Kohlendioxyd geboten. . . ." He wrote also (**39**, p. 104), "Die Pflanze assimiliert den Kohlenstoff für ihre photoenergetischen Prozesse nicht nur in Form von Kohlendioxyd mittels der Chlorophyllapparate aus der Luft, sondern auch mit dem Wurzelsystem in Form von Bikarbonaten aus dem Boden."

In 1929 MARIA BERGAMASCHI (**4**) published an account of some experiments that she had carried out in POLLACCI's laboratory, and concluded that at least some of the CO_2 assimilated by plants may be absorbed through their roots. In one series of these experiments maple, linden, camellia, and oleander plants were grown with CO_2 supplied to the root surfaces but without any CO_2 in the air surrounding the leaves, light conditions being allowed to fluctuate naturally. The leaves of these plants showed much starch in the stomatal guard cells and smaller amounts in the mesophyll.

The aerial parts were inclosed in glass chambers in which CO_2-free air was renewed several times daily, but no special arrangements were made to promote rapid transpiration; therefore the transpiration rate was probably very slow. Also, as the author pointed out, it is conceivable that the starch found in these leaves may have been wholly or partly formed from CO_2 of respiration; nor is the possibility ruled out that it may have been formed from carbohydrate partly or wholly derived directly or indirectly from food present in the plants before the beginning of the experiment.

In another series of experiments BERGAMASCHI ascertained, by sampling, the average carbon content per grain of a lot of maize seed that had been selected for uniformity from a single ear, and then plants derived from seeds of that same lot were grown under natural conditions of light and darkness, as follows: (1) Roots in nutrient solution well supplied with CO_2, leaves in CO_2-free air. (2) Roots in solution without CO_2, leaves in CO_2-free air. (3) Roots in solution well supplied with CO_2, leaves in air well supplied with CO_2. (4) Roots grown in darkness, without the possibility of photosynthesis.

At the end of an experiment period of 12–14 days, the carbon content, as a percentage of the dry weight, was ascertained for the plants of each series. The resulting percentages are as follows: (1) 43.64, (2) 39.30, (3) 75.72, (4) 34.86. The percentage carbon content for the lot of seed from which the plants had been grown was 41.54, and the differences were taken to mean that photosynthesis had been most active in the plants of group 3 (with natural light and with CO_2 supplied to both roots and leaves), but that it had occurred also in those of group 1 (with natural light and with CO_2 supplied to roots only); while absence of carbon fixation in the plants wholly deprived of CO_2 (group 2) or of light (group 4) was supposed to furnish an explanation of the fact that these groups showed lower carbon percentages than the original seeds. Unfortunately this method of computation and interpretation is misleading, for the percentage carbon content of a plant does not depend solely on the actual carbon content. Indeed it is conceivable that the percentage carbon content of a plant (reckoned on the basis of dry weight) might either increase or decrease in a growth period without regard to whether the actual carbon content increased, decreased, or was maintained. BERGAMASCHI'S published data from this experiment indicate, according to computations of our own, that the average actual carbon content per plant was in all four series less than the corresponding average per seed; that is, the experiment period was apparently not long enough, even with the plants of group 3, to permit photosynthesis, after it had begun, to make up for the initial loss of carbon that had occurred during seed germination and early growth. From the data given we have computed the following values for average actual carbon losses per plant:

(1) Plants under natural conditions of light and darkness, CO_2 supplied to roots only ..... 0.0506 mg.
(2) Plants under natural conditions of light and darkness, no CO_2 supplied ..... 0.0612 mg.
(3) Plants under natural conditions of light and darkness, CO_2 supplied to both roots and leaves ..... 0.0469 mg.
(4) Plants in darkness ..... 0.0440 mg.

It appears that the two series in which the carbon content could not have been increased from without (groups 2 and 4) are characterized by the largest and the smallest carbon loss respectively, and that the plants best supplied with CO_2 (group 3) lost somewhat more carbon than those grown in darkness (group 4). Therefore no logical conclusion concerning the problem in question seems to emerge from this experiment.

A recent paper by BARBIERI (**3**) shows how very erratic some modern writing may still be about sources of carbon for green plants. BARBIERI states flatly that plants cannot break up the carbon dioxide of the air and fix its carbon. According to his idea all of the carbon assimilated in the plant body must enter it from the soil, through the root system, either as organic matter or as mineral carbonates. We submit that *Orchid Review* was not to be congratulated on the publication of that paper.

In 1931 MILLER (**24**, p. 455) stated, "There thus appears to be good evidence that a green plant may absorb carbon dioxide from the soil and thus supplement its supply from the air." In support of this view MILLER mentions papers by GODLEWSKI, MOLL (**26, 27**), CAILLETET, MOLLIARD, MAQUENNE, POLLACCI (**32**), BREAZEALE (**8**), and BANAL (**2**), but of these only those here referred to furnish any evidence really bearing on the question before us. The remaining references are concerned with absorption of CO_2 from the free air, with emanation of CO_2 from soil to air, or with absorption of complex organic substances (rather than CO_2) from the soil.

RABER (**33**) also has recognized the possibility that the soil may supply a part of the CO_2 used by the plant, saying (**33**, p. 24) that "it is necessary to revise somewhat our ideas of the importance of carbon absorption by roots."

Some a priori considerations

If we were to suppose that all CO_2 reduced in aerially exposed green leaves must have been derived directly from the surrounding air, it would be necessary to find some sort of plausible explanation as to why some CO_2 or carbonate ion might not at times enter plant roots along with other solutes of the soil solution, being thence translocated to the leaves, and/or why CO_2 reaching the green tissues in that way might not be reduced by photosynthesis. On the basis of such a supposition it would be necessary also to find reasons why some of the CO_2 derived from respiration might not follow the same course and become reduced before escaping from the plant.

Hypotheses calculated to furnish such explanations and reasons would be very difficult to imagine and they would certainly be lacking in plausibility.

There seems to be no doubt that active cells are, in general, readily permeable to CO_2, and it has been shown repeatedly that CO_2 resulting from respiration passes out from active roots into the surrounding medium under some sets of conditions. Also, the soil solution generally contains a considerable concentration of CO_2. Therefore, as far as simple diffusion is concerned, we should expect inward diffusion of CO_2 to occur whenever CO_2 pressure is greater in the soil solution than in the root, while outward diffusion should occur whenever the pressure gradient of CO_2 is in the other direction. But water absorption by roots is probably accompanied by the entrance of the CO_2 dissolved in the absorbed water; for each liter of soil water absorbed we should expect the concomitant absorption of an amount of CO_2 equal to the amount present in that volume of soil solution before its water entered the roots. When water absorption is sufficiently rapid (as with rapid transpiration), there should be no outward diffusion of CO_2 from the roots and the rate of entrance might be much more rapid than could be accounted for by simple diffusion. Under such conditions the dissolved gas is probably carried into the roots by a mass movement of soil water. If such is not the case we must suppose that the root tissues act to prevent passage of CO_2 while allowing passage of the water in which the latter was dissolved. Such an assumption would involve a sort of filtering out of CO_2 and a resultant accumulation of CO_2 in the soil solution near the absorbing root surfaces. However, since root tissues are surely readily permeable to CO_2, there is no reason for supposing that any such "filtering out" of dissolved CO_2 occurs, although some of the mineral solutes do seem to be partially "filtered out" at times, in what is called selective absorption.

If it is agreed that roots do regularly contain CO_2 or carbonate ion (whether derived from the soil or from the respiratory process), and that cell membranes and cell walls are generally permeable to CO_2, there are just three ways by which CO_2 might move internally from roots to leaves: (1) it might simply diffuse, as solute, through the tissues, without mass flow of the solvent; (2) it might move along the paths and by the processes involved in the non-vascular transport of sugar and other organic solutes; or (3) it might be carried through xylem vessels by means of the mass flow of the transpiration stream.

(1) The process of simple solute diffusion is surely too slow to account for any considerable movement of CO_2 through such long distances as generally intervene between roots and leaves. When the distances considered are short the rate of CO_2 diffusion may be significant, however, as in the experiments of ZIJLSTRA (45), where starch was formed in leaf tissue a few centimeters beyond the region that was supplied with CO_2 from

without. Attention has been called to ZIJLSTRA'S note that gas movement in intercellular spaces was apparently important in those experiments, and it is probable that such movement might occur at a more rapid rate than solute movement if the intercellular spaces were adequate, for the mixing action of gas convection should be more pronounced in intercellular gas-filled spaces than is the similar action of liquid convection in cell cavities filled with aqueous solution. On the whole, diffusion alone may be dismissed as probably not furnishing a means for any considerable movement of CO_2 from roots to leaves, excepting when the distances involved are only a few centimeters.

(2) There is still disagreement concerning the non-vascular paths of transport of sugar and other foods in the ordinary plant, and the exact mechanism of that movement has not yet been shown clearly enough to satisfy all students. It appears, however, that the sieve tubes of the phloem offer the only path by which solutes may move from roots to leaves (or in the opposite direction) without continuous mass flow of the solution. Many writers consider these tubes as the most probable path of movement of foods, aside from movement in the transpiration stream, and MASON and MASKELL (**22, 23**) have shown that sugar and organic nitrogenous solutes surely moved in these tubes in their cotton plants. If we suppose (with CURTIS (**15**) and with MASON and MASKELL (**23**), for example) that solute movement through sieve tubes occurs partly by simple diffusion (across the sieve plates) but mostly by convection (in the supposedly streaming or rotating plasma of the several tube segments), or by some sort of creeping along liquid or liquid-solid interfaces (**14, 29**), we have at least a hypothetical scheme by which different solutes might move at much more rapid rates than would be possible by diffusion alone, although such rates would surely be greatly surpassed by the rate of flow of the transpiration stream in the xylem vessels when transpiration is rapid. This hypothesis seems to furnish the only plausible picture of a system by means of which different solutes may move in opposite directions along the same path, either at the same rate or at different rates. For example, sugar might be moving from leaf to root while CO_2 and NO_3 (**15**) might at the same time be moving from root to leaf in the same series of tubes. Movement of solutes by simple diffusion or by convection and diffusion combined may be independent of water movement excepting that of local convection. Indeed, it has not been shown that there is much transport of water through phloem at any time, although some students hypothesize a movement of solution through that tissue (**44**, and references there given). The convection hypothesis supposes that the aqueous solution of each sieve-tube section is generally being thoroughly stirred, as it were; but movement of water from one section to the next, through the plasmodesmas of the sieve plates, can

hardly be supposed to occur at rates significantly more rapid than those of water diffusion.

(3) The sap of the transpiration stream is probably never free from CO_2 that is being carried upward toward the transpiring tissues, which are generally the tissues capable of photosynthesis. MACDOUGAL, OVERTON, and SMITH (21) found that the gases of the interior of a tree trunk might contain CO_2 at high pressure. Under conditions that promote rapid transpiration, the transpiration stream probably delivers considerable amounts of CO_2 to the green leaf cells, and it is difficult to escape the supposition that all of the CO_2 thus delivered is reduced by photosynthesis when light intensity is adequate and other conditions are suitable. Unless that were true no absorption of CO_2 from the air about the foliage would occur. When illumination is inadequate (as on dark days and at night), or when the chlorophyll apparatus is inadequate (as in chlorotic leaves), CO_2 must of course be transpired, like water vapor, passing out of the leaves into the surrounding air.

No student of photosynthesis has even suggested that CO_2 formed by respiration in green leaves is not just as truly subject to reduction as is CO_2 that enters the green tissues from the air, and it would be quite unthinkable that CO_2 reaching the leaves from other regions, including the root system, is not equally subject to reduction. We cannot plausibly suppose that the chlorophyll apparatus somehow sorts internally supplied CO_2 from CO_2 absorbed from the free air and that only the latter is reduced during periods of photosynthesis. Indeed physiologists apparently agree that CO_2 absorption from the air is just the simple physical process of inward diffusion, in accordance with a concentration gradient established by the rapid removal of CO_2 from solution in the photosynthesizing cells and from the neighboring gas spaces, it being assumed that the CO_2-supplying power of the free air is continuously sufficient to maintain such a gradient. The gradient should be reversed when photosynthesis is too slow or when it does not occur, and then CO_2 should pass from leaf to surrounding air. Only when all the CO_2 reaching the chlorophyll apparatus from within the plant is reduced by photosynthesis as rapidly as it arrives is it possible for that apparatus to draw on the aerial supply of CO_2.

The body of the ordinary green plant acts as a continuous water system connecting the soil solution and the soil atmosphere below with the free air above, and the picture thus suggested is employed by many writers to furnish at least an important part of the explanation for the upward movement of water through the plant. The same picture furnishes ample ground for supposing that CO_2 absorbed from the soil, as well as that arising from respiration in roots and other darkened tissues, moves toward transpiring surfaces in an analogous manner. Under suitable conditions

for photosynthesis, some of the H_2O and some or all of the CO_2 reaching the leaves in the transpiration stream are surely reduced, thus preventing their escape into the free air in so far as they are thus fixed.

Turning to the root system: as long as CO_2 passes inward and upward out of the water-absorbing tissues as rapidly as it enters them from the soil and is produced in them by respiration, there can be no net outward movement of this substance into the soil, but when water absorption is sufficiently slow, when the concentration of CO_2 in the soil solution is low enough, and/or when the internal supply of CO_2 (as from respiration) is sufficiently rapid, then CO_2 should diffuse outward into the soil. It may be supposed that CO_2 passes from roots to soil generally only during periods of relatively slow water absorption or of rapid respiration, as in periods of slow transpiration and relatively high temperature. Not forgetting that CO_2 may to some extent diffuse outward laterally in its upward progress, it seems likely that the transpiration stream usually carries to the foliage both CO_2 derived from the soil and CO_2 produced by respiration in the cells along its course. But we should not expect any significant supply of CO_2 to reach the green tissues in this way excepting when the transpiration rate is relatively rapid. In all recorded experiments that have failed to show that some of the CO_2 reduced by photosynthesis might reach the chlorophyll apparatus *via* roots and stem, the experimental conditions have been such as to preclude rapid transpiration and rapid flow of vascular sap.

In some of MOLL'S experiments (**26, 27**) provision was made for some transpiration; the glass jar inclosing the aerial portion of the plant and confining the air about its leaves was provided with an open dish of concentrated H_2SO_4 or of strong potash lye, or a slow current of air was passed continually through the jar. These arrangements were approximately repeated in the course of our own study, to gain some idea as to the approximate magnitude of rates of transpiration that might have been possible in MOLL'S tests. Instead of a plant, a porous-porcelain atmometer was used as a source of water vapor, and it was found that only 3 or 4 gm. of water were evaporated in 24 hours. Under these test conditions, which were at least not very different from those of MOLL'S experiments, a plant could not transpire more than a very few grams of water in a day, and a portion of that water loss would naturally occur in the hours of darkness, when CO_2 delivered to the foliage from within the plant could not be reduced. Experiments in which conditions for rapid transpiration are not provided would therefore not be expected to show much transmission of CO_2 from soil through roots and stems to leaves *via* the transpiration stream. If considerable transport of CO_2 from soil to leaves through roots and stems can occur otherwise than in the transpiration stream, which has not yet been experimentally demonstrated, such transport might also be accelerated

to some extent by rapid transpiration, as well as by high concentration of CO_2 in the soil solution.

Other conditions being suitable for health and photosynthetic activity, if the concentration of CO_2 in the soil solution is high and if transpiration is rapid, we should expect a considerable but relatively small portion of the CO_2 reduced in the green tissues to be derived from the soil and from the plant's own respiratory processes, the larger portion being absorbed from the free air. But without considerable transpiration it may well be true that almost all CO_2 reduced in ordinary green leaves comes directly from the air about them in the manner commonly described.

To secure a quantitative estimate as to what fraction of the CO_2 reduced by photosynthesis might possibly come from the soil *via* roots and stem under conditions very favorable for CO_2 absorption from the soil, we may consider, for example, some alfalfa plants grown by BRIGGS and SHANTZ (9) at Akron, Colorado. For each gram of non-aqueous material produced, these plants, on the average for the season, lost by transpiration 1068 gm. of water. Let it be supposed (a) that all CO_2 brought to the leaves by the transpiration stream during daylight periods was reduced, (b) that three-fourths of the water lost by transpiration passed through the photosynthesizing tissues in those periods, and (c) that the non-aqueous material (final dry weight) of the plant may be fairly represented in this connection by the carbohydrate unit $C_6H_{10}O_5$. Then, for each gram of $C_6H_{10}O_5$ produced the CO_2 contained in 801 gm. (three-fourths of 1068 gm.) of soil solution should have been chemically reduced. If the soil solution is supposed to have contained 0.1 gm. of CO_2 per liter, then 0.08 gm. of CO_2 should be contained in 801 gm. of soil solution and that amount derived from the soil should have been reduced for each gram of $C_6H_{10}O_5$ produced. Since the production of 1 gm. of $C_6H_{10}O_5$ requires the reduction of 1.63 gm. of CO_2, 0.08 gm. of CO_2 represents 0.049 gm. (0.08/1.63) of $C_6H_{10}O_5$. Consequently, according to these various suppositions, the plants might have received from the soil about 4.9 per cent. of the CO_2 reduced by photosynthesis. Of course the respiratory processes should account for a small (but in our sense probably not negligible) additional amount of CO_2 coming to the leaves *via* the internal route, and we conclude that 95 per cent. or less of the CO_2 reduced might have been derived from the free air under the supposed conditions.

If a correction be introduced to account for the fact that the final dry weight represents somewhat less than 0.08 gm. of CO_2 per gram of non-aqueous material (since this material may contain a lower percentage of carbon than is indicated by the formula $C_6H_{10}O_5$), then the percentage of CO_2 derived from the free air should be somewhat smaller than 95, and that percentage should be still smaller if more than three-fourths of the

total water loss by transpiration occurred through the green tissues in periods of active photosynthesis. Under semi-arid conditions it is not unusual for the diurnal rate of transpiration from healthy plants to be more than three times as great as the corresponding nocturnal rate. Finally, if transpirational water loss were greater than 1068 gm. per gram of non-aqueous material produced, or if the soil solution contained more than 0.1 gm. of CO_2 per liter, then the supply of CO_2 derived from the soil would be correspondingly greater and the contribution of the free air would be correspondingly less.

Apparently no one has attempted to measure directly the CO_2 concentration of the solution about plant roots in any experiment or in the field, but measurements of the volumetric CO_2 content of soil atmosphere in the field have been made by a number of observers. We may suppose that the soil solution is in CO_2 equilibrium with the soil atmosphere, which may be considered (**10**) as under standard barometric pressure or nearly so. For a temperature of 25°, an aqueous solution of CO_2 in equilibrium with CO_2 gas under 1 atmosphere of pressure has a molar concentration of 0.0337 (**41**, p. 78), or about 1.48 gm. of CO_2 per liter. Then a soil solution containing 0.1 gm. of CO_2 per liter (as supposed in our hypothetical example) should be in CO_2 equilibrium with a soil atmosphere whose partial pressure of CO_2 is about 0.07 atmosphere; *i.e.*, the soil atmosphere corresponding to the soil solution considered in our illustration should have a volumetric CO_2 content of about 7 per cent. That value is probably higher than would represent the surface layers of soils in general, but there is no doubt that soils in which CO_2 is being rapidly produced from the decomposition of organic material (as by bacteria and fungi) would show soil atmospheres with volumetric CO_2 percentages greater than 7, especially if CO_2 diffusion directly into the free air were retarded, as in the case of rather deep soil layers with high water content or firm packing, or both. It seems not unlikely that the roots of swamp plants (such as rice growing in well manured paddies, for example) may be surrounded by solution with CO_2 concentration at least several times as great as 0.1 gm. per liter, which we have assumed. APPLEMAN (**1**) found the soil atmosphere of Maryland agricultural soils in summer, at a depth of about 10–12 cm., to have volumetric CO_2 contents as great as 5 per cent. in some instances.

From these and similar considerations the conclusion emerges that ordinary green plants with roots in soil or solution and leaves or other transpiring organs exposed to the air probably do not usually derive from the free air *all* of the CO_2 reduced by photosynthesis, but that a *portion* of that CO_2 is generally derived from the soil and, to some extent, from the plant's own respiration; also that the portion of CO_2 not derived from the free air may amount, in some instances, to at least 5 per cent. of the total

amount reduced. Such a percentage is surely not negligible for physiological science, whatever may be true for some aspects of the technology of crop production.

Some greenhouse experiments on growth in relation to CO_2 content of soil

METHODS.—In connection with our study concerning the possible photosynthetic reduction of some CO_2 derived from the soil, a series of simple greenhouse cultures was carried out to test in a preliminary and very tentative manner the relation of growth to the CO_2 content of the culture soil. Control plants were grown with the natural supply of CO_2, while the soils of the test cultures received extra CO_2. Plants of *Lupinus albus, Lycopersicum esculentum, Dracaena sanderiana,* and *Coleus blumei* were used, those of each experiment being nearly alike at the start. The experiment period, in August and September, was 24 days for *Lycopersicum* and about 45 days for each of the other forms. Frequent height measurements were made, and the average increment of shoot elongation per plant, for the whole period, was finally secured for each treatment and for each form.

The soil used was a sandy loam containing but little organic matter. Tinned sheet-iron cylinders, 19 cm. in diameter and 22 cm. high, were used as pots and these were each provided with a porous-porcelain auto-irrigator cone (**16, 19**) to maintain the mean soil moisture content nearly constant throughout the experiment period and nearly the same for all cylinders. Each cylinder was provided also with an additional irrigator cone, without water, this being buried in the soil by the side of the first. In each of the untreated cylinders the tubes from this second cone were open to the air but in each of the others the tubes served as inlet and outlet for a continuous stream of CO_2. The cultures stood about 35 cm. apart in two horizontal east-west rows, on two parallel board shelves about 25 cm. wide, 30 cm. apart, and 1 meter above the greenhouse floor. Parallel to these shelves and directly below the opening between them was a third shelf, like the others but only about 70 cm. above the floor. On this lower shelf stood the irrigator reservoir bottles. The three shelves were supported by the angle-iron frames of greenhouse benches, from which the slate tops had been removed. The cylinders that received additional CO_2 were placed alternately with the control cylinders in each row. In no case were the leaves at all crowded. Free and rapid air circulation around and among the leaves of all cultures was insured by having the ventilators in the greenhouse roof open throughout the experiment period. Thus, the CO_2 escaping from the exposed soil surface of a treated cylinder must have been rapidly disseminated in all directions, and the CO_2 concentration in the air about the foliage of the treated cultures could not have been con-

siderably higher than that of the air about the foliage of the untreated cultures. Aside from slight shade introduced by greenhouse sash and glass, light conditions were natural.

A special CO_2 generator was devised for these experiments. Two rubber-stoppered 5-gallon bottles, each with glass-tube air inlet reaching nearly to the bottom of the bottle and glass-tube outlet in the form of a siphon, are supported above an upright glass percolator 30 cm. high. The large opening of the latter (6 cm. in diameter) is closed by means of a 3-hole rubber stopper and the percolator chamber is nearly filled with quartz fragments about 0.5 cm. in diameter, into which is plunged an upright open cylindrical glass vial 3 cm. in diameter and 5 cm. high. A larger tube arranged to maintain a constant solution level is inserted in the path of each siphon tube, to maintain under practically constant pressure the outflowing solution. The two siphon tubes are eventually led downward through the stopper into the percolator and terminate side by side within the vial. One bottle is kept supplied with M/4 aqueous solution of oxalic acid, the other with a similar solution of Na_2CO_3, and the two solutions are allowed to flow slowly together and mix in the vial. Carbon dioxide is thus generated continuously and the resulting waste solution of sodium oxalate keeps the vial filled and overflowing, while the interstices among the quartz fragments in the percolator are kept filled to a depth of about 20 cm., with a gas space above. The waste solution finally escapes below through the percolator stem into a waste conduit, so bent as to maintain the solution in the percolator chamber always at the specified level, which insures the maintenance of a corresponding hydrostatic pressure upon the gas. The latter is conducted from the percolator to the first wash bottle by means of a delivery tube leading through the third hole in the rubber stopper. Rate of delivery of CO_2 is regulated by adjusting the rate of flow of the two solutions; at the lower end of each siphon tube is a porous plug of powdered quartz resting on glass wool, quartz being added or removed until the desired rate of flow is secured. In these experiments the two large bottles were replenished about once a week, the rate of flow of each solution being approximately 2 liters per day. Not quite all of the CO_2 generated escapes through the delivery outlet, for of course the waste solution has a high concentration of dissolved CO_2. To avoid some escape of this gas into the experiment room it is desirable that the waste solution be led away and discharged outside of the room. The waste conduit might be led through an opening in the greenhouse wall, emptying out of doors on the ground below.

From the generator, CO_2 was first led through a wash bottle of water, then through the first CO_2 cone in its culture cylinder, then through a second wash bottle and the second CO_2 cone, etc. It flowed through the

series continuously, by night as well as by day, at a rate of about 100 ml. per hour. From the last CO_2 cone the gas was discharged into the greenhouse, although it might better have been conducted to the outside. Thus the CO_2 content of the greenhouse air was always presumably slightly higher than that of the air out of doors, although the ventilators were open all the time; but, as has been said, there was free air circulation around all the plants, whether or not they were receiving extra CO_2 in the soil about their roots, and the apparent vigor of the plants of any culture showed no relation to their position in the greenhouse room.

By this arrangement all the CO_2 cones were kept filled with nearly pure CO_2, which diffused outward into the surrounding soil through the moist porous-porcelain wall, thus maintaining a relatively high CO_2 concentration in the soil solution of those containers that received the gas. No attempt was made to ascertain the CO_2 concentration in the soil solution of any of these culutres, but it is clear that the soil masses of the CO_2-treated cultures must have been much richer in CO_2 than were those of the controls.

RESULTS.—Data from these experiments are shown in table I. For each kind of plant and for each of the two treatments, either 1, 2, or 4 growth values are given in Roman type, followed by their average in boldface type. Each value in Roman type represents a single plant. Each culture had two plants, excepting in the case of *Dracaena*, in which there was but a single plant in each culture. The values in the right-hand column show for each kind of plant the percentage by which the average for cultures receiving extra CO_2 exceeded the corresponding control average.

The test with dark red *Coleus* was the only one failing to indicate more

TABLE I

SHOOT ELONGATION OF POTTED PLANTS AS RELATED TO CO_2 CONTENT OF THE SOIL MASS

KIND OF PLANT	INCREMENTS OF SHOOT ELONGATION		INCREASED GROWTH APPARENTLY DUE TO EXTRA CO_2 IN THE SOIL
	WITHOUT EXTRA CO_2 IN THE SOIL	WITH EXTRA CO_2 IN THE SOIL	
Coleus blumei (dark red)	8.2, 9.9: **9.1**	6.3, 8.2, 8.6, 12.8: **9.0**	−1
Coleus blumei (green)	3.9, 4.4: **4.2**	4.9, 5.7: **5.8**	26
Lycopersicum esculentum	5.2, 5.4, 5.5, 6.0: **5.5**	3.5, 7.6, 8.2, 8.9: **7.1**	29
Dracaena sanderiana	15.6: **15.6**	17.0: **17.0**	9
Lupinus albus	2.4, 3.2: **2.8**	8.2, 9.9: **9.1**	225

rapid shoot elongation in the treated cultures than in the untreated ones. The green *Coleus* showed a growth acceleration of 26 per cent. apparently related to the CO_2 treatment; and the smaller individual index from the CO_2-treated plants (4.9 cm.) is somewhat greater than the larger index from the controls (4.4 cm.). *Dracaena* (with only one control and one treated plant) indicated but little apparent effect of CO_2 treatment, growth acceleration amounting to only 9 per cent. The averages for *Lycopersicum* show growth acceleration in the treated cultures amounting to 29 per cent., but the first treated plant was clearly erratic (as was shown also by its general appearance) and the apparent CO_2 acceleration would be still greater if the first growth index of the treated group (3.5 cm.) were neglected in computing the average; if that were done the smallest individual value from the remaining three cultures (7.6 cm.) would be markedly greater than the largest individual value from the four controls (6.0 cm.). *Lupinus* showed by far the most pronounced difference between treated and untreated cultures; the extra supply of CO_2 in the soil of the former apparently accelerated growth by 225 per cent.; in this case again the smaller individual index value from the treated culture (8.2 cm.) is much greater than the larger value from the control culture (3.2 cm.).

Observations on the general appearance of the plants led to the same conclusion as is brought out by height measurements, namely, that (excepting the red *Coleus*) those plants whose roots were in soil with high CO_2 content were generally more vigorous than those whose soil received no extra CO_2, the difference being remarkably striking for *Lupinus;* less so for *Lycopersicum* and the green *Coleus;* and not very striking, but still noticeable, for *Dracaena.*

Such a limited series of results as this is to be considered with respect to the general direction of indications rather than with respect to the actual magnitudes of the apparent accelerations shown. Whatever may be the outcome of further experimental study, it is apparent that, at least under some sets of conditions, ordinary green plants may grow more vigorously if their soil solution is provided with an unusually high concentration of CO_2. Whether the growth accelerations shown by these few preliminary tests were either partially or wholly related to an extra supply of CO_2 absorbed by the roots from the soil, and transmitted to the leaves through the stems, cannot of course be definitely stated. In the tests with *Lupinus,* and perhaps also in those with the green *Coleus* and with *Lycopersicum,* it does not seem probable that all of the apparent difference between the CO_2-treated plants and the untreated ones was due merely to a difference in the rate of CO_2 supply to the green tissues; but it is of course possible that the CO_2 treatment may have been effective in other ways. For example, the supply of mineral nutrients in the soil (the so-called "plant

foods," which are not foods in any but the most careless sense) may have been somewhat greater in the treated cultures, perhaps due to more pronounced solvent action in the CO_2-treated soils; or the extra supply of CO_2 may perhaps have influenced the activity of soil organisms or nodule bacteria. These few experiments do not warrant any attempt to present here an analytical discussion of the various logical possibilities.

Summary and conclusion

1. On *a priori* grounds it appears that some of the CO_2 reduced in the photosynthesizing tissues of ordinary green plants must reach those tissues from the interior of the plant body even when transpiration is slow. A portion of the CO_2 thus supplied should arise from the plant's own respiration, but another portion should derive from direct absorption from the soil through roots. It is calculated that, with high concentration of CO_2 in the soil solution, with rapid transpiration, and with internal as well as external conditions otherwise favorable, a plant may receive from the soil as much as 5 per cent., or even more, of the CO_2 fixed in photosynthesis. There are no adequate grounds for supposing that the supply of CO_2 is always, or even generally, exclusively derived directly from the free air.

2. The transpiration stream may be considered as the most probable means of CO_2 transport from roots to leaves, although considerable transport may perhaps occur through phloem, as sugar is apparently transported either upward or downward. A few experiments have been reported as failing to show that considerable amounts of the CO_2 reduced in photosynthesis reached the leaves from within the plant, but transpiration was necessarily very slow in all these instances, because of the experimental arrangements. Satisfactorily controlled experiments with CO_2 supplied only to the plant roots and with rapid transpiration have yet to be reported.

3. A few pot experiments are described, which were carried out under greenhouse conditions in summer at Baltimore. An unusually high concentration of CO_2 was maintained in the soil mass of some pots, by diffusion from a porous-porcelain cone continuously supplied with CO_2 from a specially devised generator, and the plants so treated were generally more vigorous than the corresponding untreated ones. Growth acceleration concomitant with this CO_2 treatment was very pronounced for plants of *Lupinus albus,* less pronounced for a green form of *Coleus blumei* and for *Lycopersicum esculentum,* slight for *Dracaena sanderiana,* and absent for a dark red form of *Coleus blumei.* Attention is called to the probability that these growth responses may not have been due wholly to accelerated absorption of CO_2 from the soil, for the CO_2 treatment doubtless involved other differences between treated and untreated cultures. Arrangements were such as to promote very free air circulation around all the plants,

to avoid the possibility of a higher concentration of CO_2 in the air around the leaves of the treated plants than in the air around the leaves of the others.

LABORATORY OF PLANT PHYSIOLOGY
JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

LITERATURE CITED

1. APPLEMAN, C. O. Percentage of carbon dioxide in soil air. Soil Sci. **24**: 241–245. 1927.
2. BANAL, R. Un fertilizzante fondamentale: l'anidride carbonica. L'Italia Agric. **63**: 118–121. 1926.
3. BARBIERI, N. A. The physiological cultivation of orchids. Organic fixation of nitrogen by means of algae. Orchid Rev. **38**: 35–50. 1930.
4. BERGAMASCHI, MARIA. Sull'assorbimento del biossido di carbonio per opera delle radici e sulla sua utilizzazione nella fotosintesi clorofilliana. Atti Ist. Bot. R. Univ. Pavia. IV. **1**: 89–94; 117–149. 1929. Also Rendi conti R. Acad. Lincei. VI. **9**: 238–242. 1929.
5. BIRNER, H., and LUCANUS, B. Wasserculturversuche mit Hafer in der Agrik. chem. Versuchsst. zu Regennalde. Landw. Versuchs-St. **8**: 128–177. 1866.
6. BÖHM, JOS. Ueber Stärkebildungen in den chlorophyllkörnern. Sitzungsber. k. Akad. Wiss. Wien **73** (I): 39–66. 1876.
7. BOUSSINGAULT, J. B. Économie rurale considérée dans ses rapports avec la chimie, la physique et la météorologie. (2 vol.; pp. 648, 742) Paris. 1844. See also Die Landwirtschaft in ihren Beziehungen zur Chemie, Physik u. Meteorologie. Bd. I. Wien. 1851.
8. BREAZEALE, J. F. The absorption of carbon by the roots of plants. Jour. Agr. Res. **26**: 303–313. 1923.
9. BRIGGS, L. J., and SHANTZ, H. L. The water requirement of plants. U. S. Dept. Agr. Bur. Plant Ind. Bulls. 284, 285. 1913.
10. BUCKINGHAM, EDGAR. Contribution to our knowledge of the aeration of soils. U. S. Dept. Agr. Bur. Soils Bull. 25. 1904.
11. CERIGHELLI, M. R. Sur les échanges gazeux de la racine avec l'atmosphere. Comptes Rend. Acad. Sci. (Paris) **171**: 575–578. 1920.
12. ————————. Nouvelle recherches sur la respiration de la racine. Variation du quotient respiratoire au cours du développement. Rev. Gén. Bot. **39**: 102–112; 157–166. 1925.
13. CORENWINDER, M. B. Recherches sur l'assimilation du carbone par les feuilles des végétaux. Ann. Chim. et Phys. III. **54**: 321–356. 1858.

14. CRAFTS, A. S. Movement of organic materials in plants. Plant Physiol. **6**: 1–41. 1931.
15. CURTIS, O. F. Studies on tissues concerned in transfer of solutes in plants. Ann. Bot. **39**: 573–585. 1925.
16. DEATRICK, E. P. Porous clay auto-irrigator cones for watering potted soil and plants. Jour. Amer. Soc. Agron. **19**: 252–255. 1927.
17. EWART, A. J. Further observations upon assimilatory inhibition. Jour. Linnean Soc. Bot. **31**: 554–576. 1896.
18. FREE, E. E. The effect of aeration on the growth of buckwheat in water-cultures. Johns Hopkins Univ. Circ. March, 1917, pp. 198–199.
19. LIVINGSTON, B. E. Porous clay cones for the auto-irrigation of potted plants. Plant World **21**: 201–208. 1918.
20. MACDOUGAL, D. T. Relation of the growth of leaves and the chlorophyll function. Jour. Linnean Soc. Bot. **31**: 526–545. 1896.
21. ————, OVERTON, J. B., and SMITH, G. M. The hydrostatic-pneumatic system of certain trees. Movements of liquids and gases. Carnegie Inst. Wash. Pub. **397**: 66–67. 1929.
22. MASKELL, E. J., and MASON, T. G. Studies on the transport of nitrogenous substances in the cotton plant. III and IV. Ann. Bot. **44**: 1–29; 233–267. 1930.
23. MASON, T. G., and MASKELL, E. J. Studies on the transport of carbohydrate in the cotton plant. I and II. Ann. Bot. **42**: 189–253; 571–636. 1930.
24. MILLER, E. C. Plant physiology. pp. xxix + 900. McGraw-Hill. New York. 1931.
25. MITSCHERLICH, E. Ein Beitrag zur Kohlensäuredüngen. Landw. Jahrb. **39**: 157. 1910.
26. MOLL, J. W. Ueber den Ursprung des Kohlenstoffs der Pflanzen. Landw. Jahrb. **6**: 327–362. 1877.
27. ————. Ueber die Herkunft des Kohlenstoffs der Pflanzen. Arb. Bot. Inst. Würzburg **2** (Heft I, 1878): 105–113. 1882.
28. ————. Communication on presenting the dissertation of Mr. K. ZIJLSTRA entitled "Kohlensäuretransport in Blättern." K. Akad. Wetensch. Amsterdam. Proc. Sect. Sci. **2**: 649–668. 1909.
29. MÜNCH, E. Versuche über den Saftkreislauf. Ber. deutsch. bot. Ges. **45**: 340–356. 1927.
30. PFEFFER, W. Pflanzenphysiologie. pp. viii + 474. Leipzig. 1881.
31. ————. The physiology of plants. (Transl. by A. J. EWART). Vol. 1. pp. xii + 632. Oxford. 1900.

32. POLLACCI, G. Sul carbonio delle piante verdi. Atti Ist. Bot. Pavia. II, 17: 29–51. 1917.
33. RABER, ORAN. Principles of plant physiology. Revised ed. pp. xv + 432. Macmillan. New York. 1933.
34. SACHS, JULIUS. Vorlesungen über Pflanzen-physiologie. pp. xi + 991. Leipzig. 1882.
35. SAUSSURE, THÉODORE DE. Recherches chimiques sur la végétation. Paris. 1804.
36. SPOEHR, H. A. Photosynthesis. pp. 393. Chemical Catalog Co. New York. 1926.
37. STILES, WALTER. Photosynthesis. pp. vii + 268. Longmans, Green and Co. London. 1925.
38. STOKLASA, J. Die Bedeutung der Bakterien für die Fruchtbarkeit des Bodens. Abstracts of Proc., First Internat. Congress Soil Sci. Comm. III and IV. pp. 1–5. June, 1927.
39. ————. Dynamik und Energetik der Kohlensäureassimilation. Die Ernährung der Pflanze. Deutsch. Kalisyndikat. Berlin **25**: 97–105. 1929.
40. UNGER, F. J. A. N. Anatomie und Physiologie der Pflanzen. pp. xix + 461. Leipzig. 1855.
41. VAN NOSTRAND'S Chemical Annual. Ed. by J. C. OLSEN and T. R. LECOMPTE. 6th issue. pp. xv + 882. New York. 1926.
42. VINES, S. H. The influence of light upon the growth of leaves. Arb. Bot. Inst. Würzburg 2 (Heft I, 1878): 114–132. 1882.
43. ————. Lectures on the physiology of plants. pp. x + 710. Cambridge. 1886.
44. WOODHOUSE, E. D. Sap hydraulics. Plant Physiol. **8**: 177–202. 1932.
45. ZIJLSTRA, K. Kohlensäuretransport in Blättern. Rec. Trav. Bot. Néerlandais **6**: 99–210. 1909.

CAUSES OF BLIND WOOD IN ROSES

DONALD S. HUBBELL

(WITH TEN FIGURES)

Introduction

It is well known that many hybrid roses and also pure species produce great numbers of non-flowering branches which are commonly called "blind shoots." With some greenhouse varieties 50 per cent. or more of the average growth in one year may consist of blind shoots. These blind shoots represent a tremendous reduction in flowering wood, and because of this reduction the underlying causes of blindness are of great importance to the commercial rose grower.

The habit of blind wood formation is especially noticeable in the forced hybrid tea rose, which is a monthly blooming plant cultivated for heavy and regular flower production. The phenomenon of blindness is noticeable, however, to a lesser extent in hybrid perpetual roses and some pure species. The difference in flowering habit between the rose classes may account for the differences in blind wood production. These differences in blooming habit are exemplified by the hybrid tea rose which is never in a purely vegetative condition, and by the hybrid perpetuals and some pure species which for two-thirds of their growing season are in a vegetative state.

The production of blind wood in the rose appears to be a varietal characteristic, because with certain varieties of hybrid tea roses, regular blooming accompanied by a minimum production of blind wood occurs. Other hybrid tea varieties produce such a high percentage of blind wood that they are worthless for commercial forcing. If blind wood could be controlled some of these varieties might become valuable, and flower production could presumably be increased in many of the varieties now in commercial use.

The occurrence of blind wood on rose plants was observed many years ago. In 1859 an anonymous writer (1) stated: "Pruning no more seems to prevent free blooming than non-pruning seems to promote it. . . . When the variety Isabella Grey was placed against a warm, sunny wall, it produced thirty strong shoots each of which terminated with blind ends."

The florists' trade journals have for many years published discussions and comments on the causes of blind wood in forced rose plants. These discussions are not based on experimental data but are offered merely as a result of observations. These trade journal discussions have helped to establish the phenomenon of blindness in roses as a problem worthy of the attention of investigators in the fields of plant research.

The propagation of rose plants from blind wood was a subject for debate for many years until CORBETT (3) apparently solved the problem in 1897. After five years of research he came to the following conclusions: "(1) rose plants propagated from flowering wood gave an average yield of 29 *[illegible]* blooms per plant, whereas plants propagated from blind wood gave an average yield of 11½ flowers per plant; (2) there was little difference in rooting habit between cuttings made from blind and normal wood; (3) there was no apparent cumulative effect from the continual selection of cuttings from flowering wood, nor was there any marked degradation from continuous use of blind wood; (4) there was no difference in vegetative vigor between the two sets of plants."

CORBETT made no attempt to determine the causes of blind wood. Other writers, however, have suggested theories as to the cause. TAYLOR (6) observed in 1919 that too severe pruning produced much flowerless growth in certain rose plants.

An anonymous writer (2) in 1929 made the following assertion concerning the causes of blind wood on rose plants: "With shorter days and gradually diminishing sunlight, the plants get too little light, and this alone causes roses to produce blind wood. . . . Do not cut out blind wood but if they are pinched back they will go on and produce good flowers."

HOLMES (4) states: "A whole week in November without sun but with warm, foggy weather causes blind growth on the weaker shoots."

The writer has been unable to find any data concerning the causes of blind wood on roses, nor was CORBETT or any of the leading rose investigators able to add to the bibliography.

Materials and methods

PLOT TECHNIQUE

The plant materials required for this project were 104 grafted rose plants, which were produced by grafting 52 flowering and 52 blind cions of Mme. Butterfly on English Manetti stock; 240 spring propagated own-root rose plants of the variety Mme. Butterfly, which were used for the nitrate experiments; and 100 own-root, two-year-old rose plants of the variety Mme. Butterfly, which were used for the pruning and budding experiments. The material for analytical work was obtained from the 100 plants selected for the pruning and budding experiments.

The rose plants were grown in the rose house of the Iowa State College greenhouses. The rose house runs east and west and contains three benches, each of which is 5 feet wide, 72 feet long, and 6 inches deep. On July 1, 1931, these benches were filled with a black loam which had been removed from an uncultivated field. Ten pounds of superphosphate and one pound of muriate of potash were well mixed with the soil and added to

each 100 square feet of bench space. On July 7 the center bench and 6 feet of the north bench on the west end were used for nitrate experiments. Thirty-two linear feet of the south bench were devoted to the plants which were used for pruning, budding, and sample collections. The grafting experiment utilized 34 linear feet of the north bench.

Chemical analyses

All samples for chemical analyses were taken from a bench of two-year-old, own-root hybrid tea rose plants of the variety Mme. Butterfly. At the time of sampling, the blind and flowering shoots were four to five nodes in length and about 30 days old. Analytical methods as outlined by Loomis (5) were used. Collections were made on the 20th of each month from September, 1931, to May, 1932, inclusive.

Experimentation

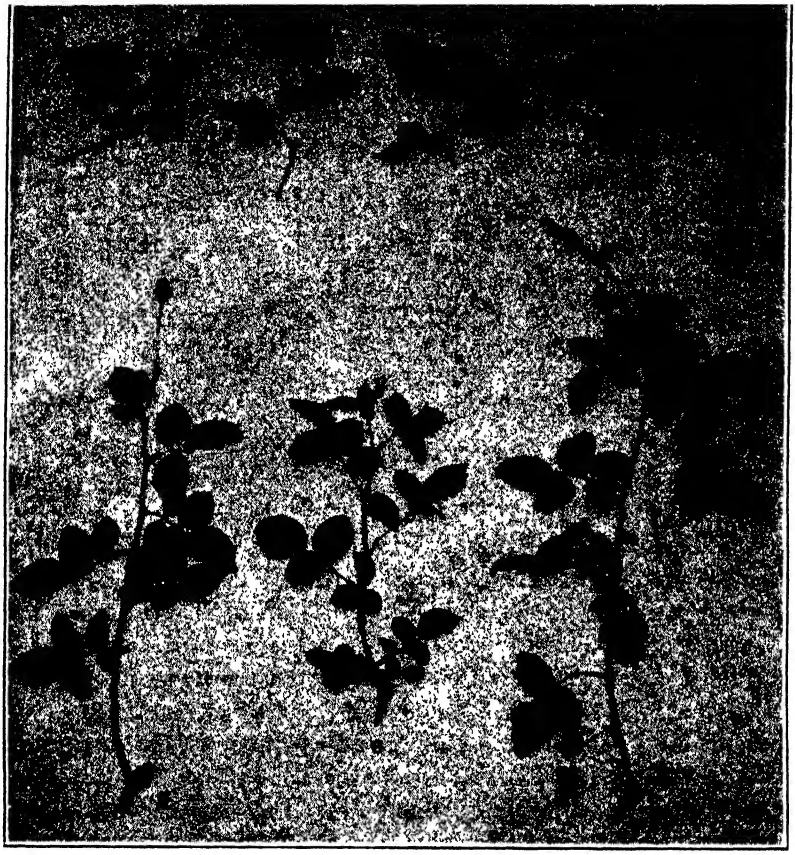
Growth habits of rose shoots

During the progress of the experiment the growth habits of blind and flowering wood on the variety Mme. Butterfly were studied. Figures 3 and 4 are illustrations of typical examples of flowering and blind rose shoots 30 days old. Shoots similar to these were used in all the experiments described in this paper. Figure 4 shows a normal flowering shoot terminated in a bud, whereas figure 3 shows a blind shoot terminated by a leaf composed of three leaflets. The flowering shoots had approximately seven nodes when they reached maturity; on the other hand, the blind shoots seldom had more than five nodes with relatively short internodes.

The blind growth of the hybrid tea differs from the active vegetative growth of the seasonal blooming rose plants such as the hybrid perpetuals, in that there is relatively little elongation of the growing point in the blind shoot after 30 days' growth. The vegetative growth of the seasonal blooming rose plant is continuous during the growing season. This active growth results in the formation of a long shoot with an indefinite number of nodes and with the terminal bud inclosed in a rosette of leaves.

In the hybrid tea rose plant of the variety Mme. Butterfly one to three shoots may develop from the upper axillary bud at the time when flowering should occur. These new shoots may continue blind or occasionally one may develop into a flowering shoot. If one of the three shoots produces a terminal bud, it may or may not develop into a normal flower. In the event that no flower formation occurs, blind growth continues intermittently or until a flowering shoot is produced. Figure 7 shows a shoot which has terminated in blind ends for three consecutive breaks from the axillary bud just behind the blind terminal.

As a rule flowering shoots have a higher percentage of moisture and



FIGS. 1–7. Fig. 1, blind shoot 55 days old, produced by budding a bud from a flowering shoot on a blind shoot. Fig. 2, flowering shoot 55 days old, produced by budding a bud from a flowering shoot on a blind shoot. Fig. 3, typical blind shoot 30 days old. Fig. 4, typical flowering shoot 30 days old. Fig. 5, flowering shoot 55 days old, produced by budding a bud from a flowering shoot on a flowering shoot. Fig. 6, flowering shoot 55 days old, produced by budding a bud from a blind shoot on a flowering shoot. Fig. 7, blind shoot approximately six months old.

appear to be more succulent and immature than the blind shoots. On the other hand, blind shoots are more slender and elastic than the normal shoots. It is the opinion of rose growers that at least 60 per cent. of the blind shoots will produce flowers in the spring of the year.

It is a common practice to bud the continuous blooming hybrid teas on seasonal blooming rose plants such as the hybrid perpetual. In most cases the hybrid tea bud retains its everblooming habit. This relationship between the bud and the stock indicates that a bud retains its flowering characteristics regardless of the stock, provided the two are compatible.

Table I shows some of the observable differences between blind and flowering shoots of the variety Mme. Butterfly. The figures show the average differences between the two types of shoots for the forcing season and

TABLE I

DIFFERENCES BETWEEN FLOWERING AND BLIND SHOOTS

MATERIAL	LEAF SURFACE	MOISTURE	DRY MATTER	GREEN WEIGHTS PER STEM
	sq. in.	%	%	*gm.*
Normal stems	...	86	14	1.32
Blind stems	.	76	24	0.82
Normal leaf	12	84	16	2.50
Blind leaf	16	79	21	3.10

are expressed as differences between single shoots approximately 30 days old. The flowering shoot at 30 days of age is very immature and will approximately treble its weight and double its leaf surface by the time it reaches maturity. The blind shoot, on the other hand, has usually reached the height of its development when it is 30 days old. The average length of a mature flowering stem is 14 inches and its diameter 6 mm., while the average length of a 30-day-old flowering stem is only 6 inches with a diam-

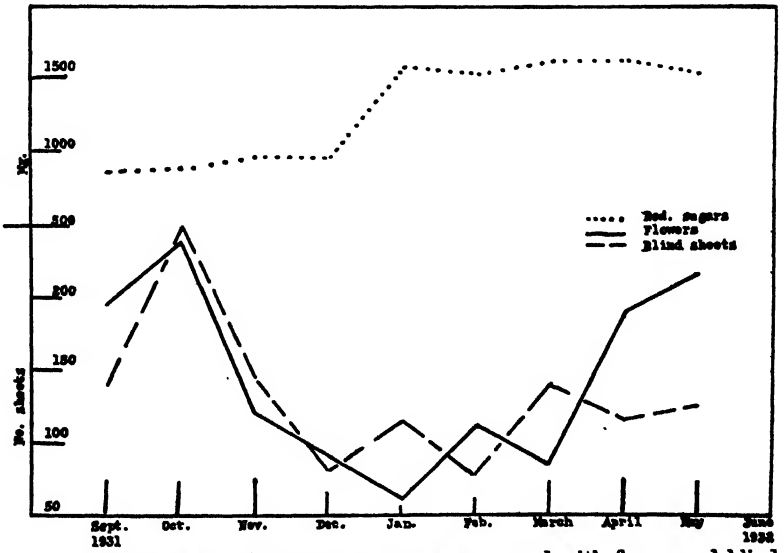


FIG. 8. Seasonal trend of reducing sugars as compared with flower and blind shoot production.

eter of 4 mm. The mature blind shoot averages 4 inches in length and has an approximate diameter of 3 mm.

The figures in table I indicate that blind and flowering shoots of an immature age differ greatly in the percentage of moisture which they contain, as well as in leaf surface. The blind shoots have approximately one and one-half times as much leaf surface as the normal shoots when they are 30 days old, and at the same time contain 10 per cent. less moisture.

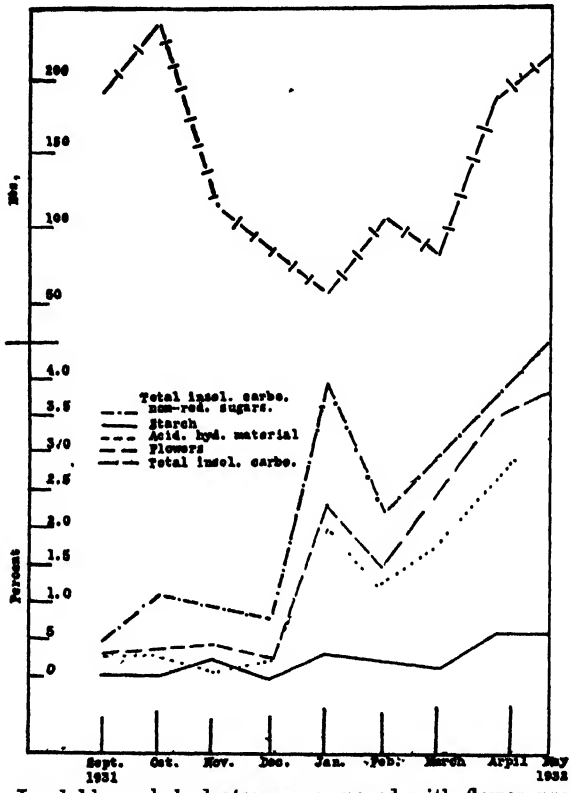


FIG. 9. Insoluble carbohydrates as compared with flower production.

The normal growth of the hybrid tea rose is of a determinate type. The nodes of 53 mature flowering stems were counted and found to average 7.3 nodes per stem. The average number of nodes on 38 immature flowering stems 30 days old was 7.1 nodes per stem. It is evident that the number of nodes present in the mature flowering stem is determined early in the growth of the shoot. Node counts made on blind shoots at 30 days of age averaged 4.5 nodes per stem and the average number of nodes for blind stems just breaking into growth was 4.9 nodes.

These data indicate that elongation of the blind shoot is stopped by the

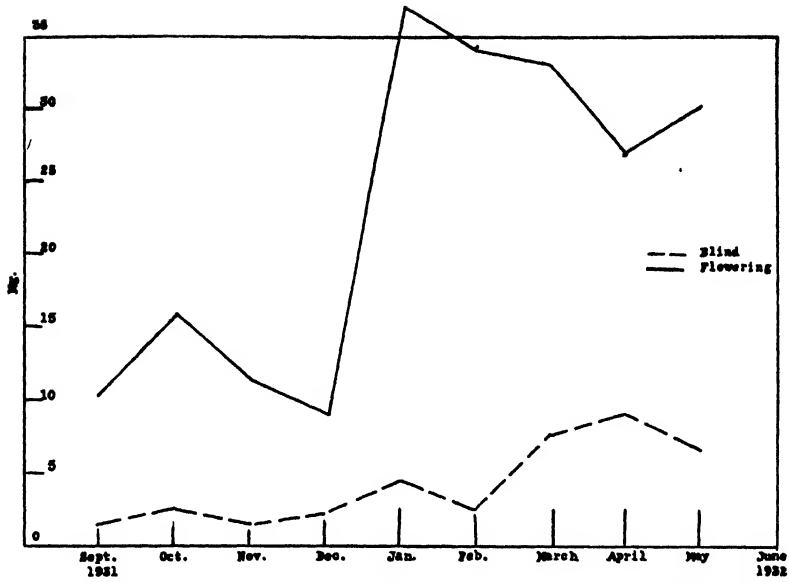


FIG. 10. C/N relationship between blind and flowering wood expressed as mg. reducing sugar per mg. non-colloidal nitrogen.

failure of the terminal flower bud to develop. The blind stem remains inactive for approximately 30 days and the first axillary bud from the tip then acts as a vegetative terminal and growth is resumed. The last two nodes on the mature flowering stem are not in prominence until they near maturity, and the attached leaves are usually in the form of misshapen bracts rather than normal leaves with from three to five leaflets. These last two nodes are not in evidence on the blind stems, since they usually have five nodes accompanied by normal leaves. It is presumed that when flower formation in the blind shoot was stopped by certain unknown factors, the formation of the last two nodes was suppressed at the same time.

BLIND WOOD AS A PHYSIOLOGICAL RATHER THAN AS A GENETIC OR PATHOLOGICAL CONDITION

GRAFTING EXPERIMENTS.—CORBETT (3) suggests that blindness in roses is an inherited characteristic. The basis for this suggestion was that plants produced from cuttings of blind wood yielded fewer flowers than plants produced from cuttings of flowering wood. Since his results were obtained from own-root plants, the writer decided to determine whether the same effects could be obtained by grafting flowering and blind cions on English Manetti.

Blind and flowering cions from Mme. Butterfly rose plants were grafted on English Manetti stock on February 1, 1931. These grafted

plants were carried in 2.5-inch rose pots until May 1, when they were shifted to 4-inch pots. On July 7, 52 uniform plants of each type of cion were selected and benched. From the time they were benched the plants were given the customary commercial rose culture. They received applications of manure water in September and October, and in February the plants were given a top dressing of fresh cow manure which was supplemented with feedings of manure water once a month until June 1. The flowers were cut and counted daily, and the blind shoots were counted at the end of each month. After the count was made the blind shoots were pinched back to the first bud back of the tip.

The yields of flowers and blind shoots of plants grafted with blind and flowering cion wood are given in table II. The main differences between

TABLE II

COMPARISON OF BLIND AND FLOWERING SHOOT PRODUCTION FROM CIONS OF BLIND AND FLOWERING WOOD

DATE	FLOWERING CIONS				BLIND CIONS			
	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND
1931		%		%		%		%
Sept.	194	58	140	42	167	51	160	49
Oct.	239	49	251	51	237	55	194	45
Nov.	120	45	146	55	129	63	77	37
Dec.	90	52	82	48	83	61	52	38
1932								
Jan.	61	35	113	65	71	43	94	57
Feb.	108	57	81	43	95	57	72	43
March	85	38	138	62	98	59	69	41
April	188	62	115	38	155	62	97	38
May	218	63	126	37	234	69	106	31
June	283	68	132	32	278	71	112	29

the two graft types are in total shoot production and in the production of blind wood. The plants derived from flowering cion wood produced 39 more flowers and 291 more blind shoots than the plants derived from blind cions. The differences in flower production are not significant, but the differences in total shoot production are. The increase in total shoot production in the plants produced from flowering cion wood indicates that these plants were superior in vegetative vigor. The differences in vegetative vigor between the plants produced from the two types of cion wood could have been caused by the differences in cion diameter. The average diameter of the blind cion wood was 3 mm., whereas the average diameter of the flowering cion wood was 6 mm. The small diameter of the blind cion wood possibly caused more of a callous constriction than did the union

between the larger flowering cion and its stock. This constriction could have affected the plants from the blind cion in the same manner as girdling would have done; that is, a carbohydrate reserve was built up above the graft union, which stimulated flower production over vegetative growth.

It is evident from the data (table II) that there were no inherited effects from the use of blind cions in so far as the production of blind wood was concerned, nor were there any inherited effects on flower production from the use of flowering cion wood.

PRUNING AND BUDDING EXPERIMENTS.—The purpose of these experiments was to determine whether or not blindness occurred within the axillary buds of the blind shoot.

One hundred own-root, two-year-old rose plants were used for pruning experiments. The work was started in the fall of 1930 and completed June 1, 1932. Blind and flowering shoots which were approximately 30 days old were pruned to the first, second, or third axillary bud from the tip. The prunings were made whenever there were any available shoots of the proper age. For the budding experiments, buds from a 30-day-old blind shoot were budded on to mature flowering stems and buds from mature flowering stems were budded on to blind shoots. To check the results of the budding practices, normal buds were budded into normal shoots.

The results from the pruning and budding experiments are recorded in table III. The data indicate that blindness does not originate in the axillary bud but is the result of conditions arising after the bud starts to grow. The data also indicate that blindness in the bud is influenced to a marked extent by its stock.

From 200 flowering shoots, pinched to a first, second, or third axillary bud, only 3 per cent. produced blind shoots; whereas when 400 blind shoots

TABLE III

RESPONSE OF BLIND AND FLOWERING ROSE SHOOTS TO BUDDING AND PRUNING EXPERIMENTS

TREATMENT	FLOWERING SHOOTS PRODUCED	PERCENTAGE	BLIND SHOOTS PRODUCED	PERCENTAGE
		%		%
Flowering buds budded in flowering shoots	81	85.36	14	14.63
Flowering buds budded in blind shoots	6	12.50	42	87.50
Blind buds budded in flowering shoots	204	87.93	28	12.08
Flowering shoots pruned back	186	97.38	5	2.61
Blind shoots pruned back	11	2.89	380	97.10

were pinched, 97 per cent. of the new shoots produced were blind. When 100 axillary buds from flowering shoots were budded on mature stems, 15 per cent. of the new shoots formed were blind, but when 48 normal buds were budded on blind shoots, 87 per cent. of the buds were blind. Of the 232 buds taken from blind shoots and budded on to normal shoots, only 12 per cent. were blind. The 48 buds which were budded on blind shoots represent the number of buds which produced shoots. The number of buds set in blind shoots was 205. It is evident that since only 23 per cent. of the buds formed a union with the stock, there was some form of incompatibility between the two. This incompatibility was no doubt the fault of the stock, since the buds formed good unions with the flowering wood.

The preceding data indicate that blindness is a result of the stock and does not necessarily occur in the bud.[1,2] This point is illustrated by the fact that in most cases when a bud is forced into growth on a blind stem it remains blind. If, however, the bud is placed on a normal stem it produces a flower. It appears that the bud is influenced by the stock, and this fact, taken in connection with the results of the chemical analyses, indicates that blindness is a nutritional factor.

Effects of sodium nitrate applications on flower and blind wood production

Preliminary experiments indicated that the nitrogen content varied considerably between blind and flowering wood. With this variation in mind, rose plants were given varying applications of sodium nitrate to determine whether or not the quantity of soil nitrate affected the formation of blind shoots.

Two hundred and forty own-root, spring-propagated rose plants were divided among plots of high, medium, and low nitrogen content. Plots consisted of 34 square feet of bench area, separated by $1'' \times 12''$ cypress boards. Twenty carefully selected plants were planted in each plot on July 7 with alternating treatments of high, medium, and low nitrate concentrations. This plot arrangement for each treatment allowed 80 plants to be well distributed through the length of the rose house.

The high nitrogen plots received 6 pounds of sodium nitrate for every

[1] The time of flower initiation has not been determined for the rose. For this reason it was not known in the budding experiments whether the bud used was blind, flowering, or undifferentiated at the time of budding. The lack of such knowledge probably affected the budding results to some extent. The data indicate, however, that the axillary buds will produce flowering shoots provided they are grown on a flowering stem.

[2] Hubbell, D. S. A morphological study of blind and flowering rose shoots, with special reference to flower-bud differentiation. Jour. Agr. Res. 48: 91–95. 1934. This paper shows that differentiation starts to take place eight days after an axillary bud starts growth. Hence the buds in question here were in a state of undifferentiation.

TABLE IV

BLIND AND FLOWERING SHOOT PRODUCTION FROM HIGH, MEDIUM, AND LOW NITROGEN PLOTS

DATE	HIGH NITROGEN PLOT				MEDIUM NITROGEN PLOT				LOW NITROGEN PLOT			
	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND	FLOWERS	PERCENTAGE FLOWERS	BLIND SHOOTS	PERCENTAGE BLIND
		%		%		%		%		%		%
Sept.	99	47.82	108	52.17	170	45.69	202	54.30	191	45.15	232	54.84
Oct.	154	53.65	133	46.34	219	47.50	242	53.49	238	40.43	353	59.56
Nov.	113	55.39	91	44.60	172	60.13	114	39.86	169	51.68	158	48.31
Dec.	81	57.44	60	42.55	80	51.61	75	48.38	95	45.26	115	54.76
Jan.	53	40.45	78	59.54	60	30.76	135	69.23	52	23.00	174	77.00
Feb.	96	64.00	54	36.00	111	54.67	92	45.32	95	43.37	124	56.62
March	138	64.18	77	55.81	151	50.50	148	49.49	128	41.10	181	58.89
April	144	56.25	112	43.75	180	61.39	114	38.60	119	50.00	119	50.00
May	238	72.79	89	27.21	267	65.83	139	34.17	117	52.24	107	47.76
June	301	75.82	95	24.18	332	78.07	95	21.93	167	66.82	83	33.18
	1417	61.24	897	38.76	1742	56.23	1356	43.77	1371	45.45	1646	54.55

100 square feet of bench space during the year. The nitrate was applied dry at the rate of 2 pounds per 100 square feet in the months of July, December, and February and was watered into the soil immediately after its application. The medium nitrogen plots received a total of 3 pounds of sodium nitrate, applied at the same time and in the same manner as the high nitrogen plots. The low nitrogen plots received no nitrate fertilizer.

Beginning September 1, 1931, the number of blind shoots and flowers produced on each plot was counted. The flowers were counted daily and the blind shoots were counted at the end of each month until June 30, 1932. After the count was made the blind shoots were pinched back to the first bud from the tip.

Table IV shows a correlation between the nitrate content of the soil and blind and flowering wood production. In all three fertilizer treatments the low point in total shoot production occurred in December, January, and February.

The high nitrogen plot produced the fewest flowers and the fewest blind shoots for the season. This increase in total shoot production was apparently the result of an excess of nitrate of soda. Evidence of nitrate burning was noted on the foliage throughout the season. The average percentage of blindness for the season was only 39 per cent. The medium nitrogen plot produced the most flowers and second to the lowest number of blind shoots for the season. The average percentage of blindness for the season was 44 per cent., or 5 per cent. more than the high nitrogen plot. The low nitrogen plot was lower than the medium nitrogen plot in flower production but higher in blind shoot production. The average percentage of blind shoots for the season for the low nitrogen plot was 54 per cent., or 11 per cent. higher than the medium nitrogen plot and 16 per cent. higher than the high nitrogen plot.

The data indicate that when nitrogen is applied to the soil in the form of a nitrate it has a definite effect on the production of blind wood. An increase in nitrate supply decreased blind shoot production.

The medium nitrogen plots were the only ones which produced normal and healthy plants for the entire season. The plants within the high nitrogen plots suffered from nitrate burning while they were becoming established. The low nitrogen plot produced apparently normal plants until March, when yellowing of the foliage and decreased growth gave evidence of nitrate starvation.

Blind wood as a seasonal phenomenon

Effect of illumination on blind and flowering shoot production.—Table V shows the number of blossoms and blind shoots formed for each month from September to June inclusive. The blind shoots pro-

duced for the year were almost equal in number to the flowering shoots. When there is a decrease in flower production there is a proportionate decrease in the number of blind shoots, and vice versa. The greatest decrease in total shoot production occurs during the months from November to February inclusive, and it was during these months that the hours of monthly illumination were at their minimum. It is evident from table V that decreased monthly illumination did not increase the percentages of blind wood, although decreased illumination did decrease flower production. These figures indicate that the total number of hours of sunshine may be correlated with total shoot production, in that decreased illumination results in decreased total shoot production and increased illumination brings about an increase in total shoot production. It is apparent that increased monthly illumination in the spring increased flower production over blind shoot production, and decreased monthly illumination in the fall had an equally depressing effect on both flower and blind shoot production. The correlation between sunshine and flowering is improved by lagging the light figures 30 days. This improved correlation indicates that one month's flowers are determined by the sunlight during the period of maximum development of the shoot, which occurs about 30 days before flowering. Total growth thus appears to be correlated with light conditions suitable for photosynthesis, but the April drop in percentage of blind wood remains to be explained.

SEASONAL CHANGES IN CARBOHYDRATE RESERVE OF NORMAL ROSE SHOOTS.—Inasmuch as it is generally assumed that the reserve carbohy-

TABLE V

COMPARISON BETWEEN TOTAL MONTHLY HOURS OF SUNSHINE AND BLIND AND FLOWERING SHOOT PRODUCTION

DATE	MONTHLY SUNSHINE	FLOWERING SHOOTS PRODUCED	PERCENTAGE FLOWERING SHOOTS	BLIND SHOOTS PRODUCED	PERCENTAGE BLIND SHOOTS
1931	*hrs.*		%		%
Sept.	225	194	58	140	42
Oct.	179	239	49	251	51
Nov.	108	120	45	146	55
Dec.	116	90	52	82	48
1932					
Jan.	121	61	35	113	65
Feb.	184	108	57	81	43
March	205	85	38	138	62
April	228	188	62	115	38
May	315	218	63	126	37
June	296	283	68	132	32

drate supply is associated with flower formation, it is of interest to note the reserve carbohydrate relationships which exist between the flowering rose shoots and the periods of monthly bloom.

The curve represented in figure 8 indicates the trend of flower production in relation to the reducing sugars present in the flowering shoot. The trend is toward increased reducing sugars from fall to spring; however, there are fluctuations in the reducing sugar curve which occur simultaneously with fluctuations in flower production. When there is a decrease in reducing sugars there is an increase in flower formation, and when there is a decrease in flower production there is an increase in reducing sugars.

The curve trends for the alcohol-insoluble carbohydrates are different from those of the reducing sugar curve. The curves in figure 9 show that the individual insoluble carbohydrates have the same trend, and because of this only the curve for total insoluble carbohydrate plus non-reducing sugars will be considered. In general the curves indicate that an increase in alcohol-insoluble carbohydrate is associated with an increase in flowering. A very decided variation from this trend occurred in January when flower production was at its lowest. This low point in flower production was emphasized by a decided increase in insoluble carbohydrates, an accumulation which may have been due to the use of shoots more than 30 days of age. Since growth is retarded during this season of the year, as is indicated in figure 9, the shoots may have appeared to be 30 days of age and still be a week or more older, owing to retarded growth. This sudden check in growth could be responsible for the carbohydrate accumulations in January. In February when flower production began to increase there was a decided decrease in insoluble carbohydrates. From February on the general trend of the curve indicates an increase in insoluble carbohydrate in proportion to the increase in flower production.

It is interesting to note that, previous to the decided increase of insoluble carbohydrate in January, the total shoot production was in steady decline, whereas the quantities of insoluble carbohydrate remained almost constant. It is evident from the data that the decrease in vegetative activity which occurred in January was associated with a decided increase in total carbohydrate. In this connection it is of interest to observe that the non-colloidal nitrogen figures in table VI indicate that there is an increase in non-colloidal nitrogen in December and a sudden decrease in January. The decrease in non-colloidal nitrogen together with the increase in insoluble carbohydrate gives evidence of inactivity in the normal rose shoots in January and correlates well with the decrease in flower production.

Chemical differences in flowering and blind shoots

Differences in colloidal and non-colloidal nitrogen.—The great differences in non-colloidal nitrogen noted in tables VI and VII between flowering shoots and blind shoots appear significant. The blind stems contain two or three times as much non-colloidal nitrogen as the flowering stems, whereas the differences in non-colloidal nitrogen content in the leaves are small. Since non-colloidal nitrogen is considered as the most active form, these data suggest that the low non-colloidal content in the flowering stem may have been brought about through its utilization by the actively growing tissue. The accumulation of non-colloidal nitrogen in the blind shoot indicates that there is a surplus of active nitrogenous material in a relatively inactive tissue. Since the blind tissue is making little or no growth at the end of 30 days, there is no use for the nitrogenous material and an accumulation results. The figures show a direct correlation between flower production and the non-colloidal nitrogen content of blind wood. It appears that an increase in non-colloidal nitrogen is associated with flowering. This association is probably due to the flowering of blind shoots, and it is especially noticeable during the spring months when flower production is high and blind shoot production is low.

TABLE VI

Colloidal and non-colloidal nitrogen in rose stems expressed as mg. per 100 gm. fresh weight

Material	Date of sample collection	Non-colloidal nitrogen	Colloidal nitrogen	Total nitrogen
	1931	*mg.*	*mg.*	*mg.*
Flowering stem	May	62	396	458
Blind stem		138	311	449
Flowering stem	June	97	317	414
Blind stem		180	318	498
Flowering stem	Sept.	81	299	381
Blind stem		195	289	484
Flowering stem	Oct.	55	236	294
Blind stem		181	253	434
Flowering stem	Nov.	83	364	447
Blind stem		163	240	403
Flowering stem	Dec.	104	266	370
Blind stem		159	262	422
	1932			
Flowering stem	Jan.	45	178	233
Blind stem		131	322	454
Flowering stem	Feb.	45	221	266
Blind stem		156	262	418
Flowering stem	March	51	221	273
Blind stem		121	230	351
Flowering stem	April	62	293	356
Blind stem		138	252	391
Flowering stem	May	52	446	498
Blind stem		159	265	425

The data in tables VI and VII show fluctuations in colloidal nitrogen content for both flowering and blind tissue. There are no apparent correlations between colloidal nitrogen content and blind shoot production. The fluctuations apparent in tables VI and VII remain unexplained. In November and January the increase was in favor of the flowering stems, but in January the blind stems showed an increase in colloidal nitrogen.

TABLE VII

COLLOIDAL AND NON-COLLOIDAL NITROGEN IN ROSE LEAVES EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL	DATE OF SAMPLE COLLECTION	NON-COLLOIDAL NITROGEN	COLLOIDAL NITROGEN	TOTAL NITROGEN
	1931	*mg.*	*mg.*	*mg.*
Flowering stem	May	52	1086	1139
Blind stem		38	1238	1276
Flowering stem	June	38	916	954
Blind stem		34	1020	1058
Flowering stem	Sept.	38	490	528
Blind stem		58	535	594
Flowering stem	Oct.	27	480	508
Blind stem		17	658	675
Flowering stem	Nov.	51	778	830
Blind stem		41	739	780
Flowering stem	Dec.	48	681	729
Blind stem		10	692	702
	1932			
Flowering stem	Jan.	62	677	739
Blind stem		45	677	722
Flowering stem	Feb.	38	633	671
Blind stem		31	740	771
Flowering stem	March	51	646	698
Blind stem		45	665	710
Flowering stem	April	34	942	977
Blind stem		34	877	912
Flowering stem	May	69	843	912
Blind stem		55	932	988

DIFFERENCES IN SOLUBLE CARBOHYDRATES.—The data for reducing sugars in stems and leaves of blind and flowering rose shoots are presented in tables VIII and IX. The percentage of reducing sugars in the flowering stems was from two to four times greater than the percentage in the blind stems. The percentages of reducing sugars for each type of stem remained constant from September to January, when a sudden increase occurred which was maintained to the month of June. It is apparent that when large quantities of reducing sugars occur in the leaves an increase is also noted in the stems. In all cases the leaves from flowering shoots were higher in reducing sugars than were the leaves from blind shoots. The data indicate that blind shoots and low reducing sugar content are associ-

ated. This relationship might be expected, because reducing sugars represent the active forms of carbohydrates and where they are present in relatively large quantities flower formation should be favored. In the case of blind shoots where there is no flower formed, reducing sugars are not present in large quantities. In the spring of the year, when flower formation is high and blind shoot production is low, there is an increase in reducing sugars in the blind shoot. When this is associated with the non-colloidal nitrogen trend, it appears that increased non-colloidal nitrogen and increased reducing sugars in blind tissue indicate an existing condition favorable to flower formation. Since this condition appears in the spring, it may be related to the observation that many blind shoots produce flowers in the spring, thus increasing total flower production and decreasing blind shoot production.

TABLE VIII

SOLUBLE AND INSOLUBLE CARBOHYDRATE IN ROSE STEMS EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL (STEM)	DATE OF SAMPLE COLLECTION	REDUCING SUGARS	NON-REDUCING SUGARS	TOTAL SUGARS	STARCH	ACID HYDROLYZABLE	TOTAL CARBOHYDRATE
	1931	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Flowering	May	1360	174	1534	.....	2480	4014
Blind		605	583	1188	.....	4318	5506
Flowering	June	2200	308	2508	.....	2757	5265
Blind		450	754	1204	.....	4970	6174
Flowering	Sept.	858	223	1081	.....	337	1418
Blind		281	926	1207	.....	309	1516
Flowering	Oct.	880	705	1585	.....	403	1988
Blind		420	700	1120	.....	445	1565
Flowering	Nov.	965	700	1665	218	175	2058
Blind		315	470	785	.....	375	1160
Flowering	Dec.	965	637	1638	.....	249	1887
Blind		360	1058	1358	.....	425	1783
	1932						
Flowering	Jan.	1580	1695	3275	311	2078	5664
Blind		555	950	1505	343	3052	4900
Flowering	Feb.	1535	782	2317	227	1375	3919
Blind		420	1185	1505	75	2997	4577
Flowering	March	1705	1038	2743	132	1841	4716
Blind		880	1051	1931	39	3228	5198
Flowering	April	1720	275	1995	607	2944	5546
Blind		1215	780	1940	917	4216	7073
Flowering	May	1570	691	2261	601	3287	6149
Blind		1073	1431	2504	834	4034	7372

The non-reducing sugar content for flowering and blind stems and leaves is given in tables VIII and IX and shows excessive fluctuations and variations between the two types of tissue. Leaves from blind shoots had higher percentages of non-reducing sugar than had leaves from flowering shoots.

TABLE IX

SOLUBLE AND INSOLUBLE CARBOHYDRATE IN ROSE LEAVES EXPRESSED AS MG. PER 100 GM. FRESH WEIGHT

MATERIAL (STEM)	DATE OF SAMPLE COLLECTION	REDUCING SUGARS	NON-REDUCING SUGARS	TOTAL SUGARS	STARCH	ACID-HYDROLYZABLE MATERIAL
	1931	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Flowering	May	2025	780	2805	.....	3220
Blind		1240	1350	2590	.....	4464
Flowering	June	4110	196	4306	.....	4031
Blind		1640	536	3176	.....	4736
Flowering	Sept.	435	1429	1864	.....	216
Blind		440	767	1207	.....	220
Flowering	Oct.	435	772	1207	.....	313
Blind		325	1140	1465	.....	293
Flowering	Nov.	385	1413	1798	76	120
Blind		300	992	1292	.....	113
Flowering	Dec.	480	905	1385	.....	232
Blind		465	1323	1788	.....	120
	1932					
Flowering	Jan.	795	389	1184	642	1645
Blind		785	1712	2317	700	1353
Flowering	Feb.	890	1707	2597	135	1281
Blind		570	2227	2797	.....	1317
Flowering	March	1195	1762	2957	325	976
Blind		1225	1850	3075	.....	1880
Flowering	April	880	583	1463	623	2042
Blind		815	1712	2527	640	3225
Flowering	May	965	1748	2713	666	1920
Blind		750	2109	2859	682	2626

The presence of these inactive sugars in blind tissue suggests that they are present as reserves. These reserves are associated with conditions of inactive growth and reduced flower formation. These conditions are not favorable to carbohydrate utilization and an accumulation results.

DIFFERENCES IN POLYSACCHARIDES.—The percentages indicated in tables VIII and IX show great differences in polysaccharide content between blind and flowering shoots. Both starch and acid-hydrolyzable material increased in the spring of the year in both types of tissue. The blind shoots were consistently higher in all polysaccharides. Table VII shows the seasonal trend for acid-hydrolyzable material in blind and flowering shoots. The blind stems increased from 0.3 per cent. in September to 3 per cent. in May. Again, as was the case in non-reducing sugars, there is an apparent increase in stored material associated with inactive growth and non-fruitfulness. A sudden increase in acid-hydrolyzable substances occurs in January and continues through May. There was no evident decrease through the heavy flowering season except in the fall. From September to January the leaves from flowering shoots had a higher acid-hydrolyzable content than had the

leaves from blind shoots. From January to June these conditions were reversed and the blind leaves had higher amounts of acid-hydrolyzable material than had the normal leaves. This reversal in the leaves was possibly due to the conversion of acid-hydrolyzable substances in normal leaves into translocation material. This converted material is probably represented by the increased reducing sugars present in flowering stems and leaves. Since there is no flower formation in the blind shoots, the reserve products are not utilized to the fullest extent and an accumulation is thus brought about. It is apparent from table VIII that the total carbohydrate is continually higher in the blind shoot throughout the spring. This increase takes place in January and continues through June.

CORRELATIONS BETWEEN THE SUGAR AND NITROGEN RATIOS AND FLOWERING.—A study of the curves presented in figure 10 shows that the balance between non-colloidal nitrogen and reducing sugars varies greatly for the two types of tissue. This relationship was suggested in a previous discussion under reducing sugars. It is obvious that the ratio of sugar to nitrogen is much greater in the flowering shoot than it is in the blind shoot. This suggests that the sugar value is a limiting factor in flower formation. This point is emphasized by the rise in carbohydrate relationship to nitrogen during late winter and spring. The rise is also noted in the blind shoot, although the ratio here scarcely reaches the lowest level of the ratio found in the flowering shoots. The highest level of the ratio in blind tissue occurs in the spring when blind shoot production decreases and flower formation increases. This rise in the ratio and the drop in blind wood production indicate that the increased ratio of sugar to nitrogen is correlated with flowering in blind shoots, and may be responsible for the percentage decrease in blind wood and increase in flowering. On the other hand, the increased percentage of flowering in nitrated plots and the positive correlation between the high non-colloidal nitrogen in blind wood and total flower production on the plants indicate that no simple high nitrogen, low carbohydrate relationship is responsible for the production of the vegetative blind shoots.

Discussion

Although the preceding data do not entirely solve the problem of blindness in roses, it is felt that they do yield some fundamental information which appears to have a direct bearing on the causes of blindness in the rose plant.

From the experimental data it is evident that the rose shoot has a determinate type of growth with a fairly definite number of nodes formed previous to or at the time of flower initiation. Elongation of the blind shoot ceases when the last node makes its appearance, whereas elongation con-

tinues in the flowering shoot until the flower has reached maturity. The flowering shoot has on an average two more nodes than the blind shoot, and it is assumed that the necrosis of the flower bud in blind wood also prevents the formation of the last two nodes in the blind shoot.

The physiological behavior of blind shoots gives additional information as to the causes of blind wood. The statement of CORBETT (3) that "tendencies manifested in a branch are perpetuated from generation to generation in plants propagated asexually" does not necessarily hold true with grafting processes. It was found that plants produced by grafting blind cions on English Manetti gave little or no evidence of inferiority over plants which were produced from flowering wood. These results indicate that the tendency of roses to produce blind wood is not inherited but is a growth expression dependent upon the vigor of the stock. The fact that 97 per cent. of the pruned blind shoots produced blind shoots is evidence against the practice of pruning blind shoots as a means of increasing productiveness in roses. The budding experiments indicate that the limitations of flower formation are based on the activity of stock and not on the impotency of the bud. Since 88 per cent. of the buds taken from blind shoots produced flowers when budded on to normal stocks, it seems reasonable to assume that blind shoots are capable of producing flowers in so far as the buds are concerned. A summation of the results from the pruning and budding experiments indicates that blindness is a result of the physiological condition of the stock and that selection within a clone does not affect the proportions of blind and flowering shoots.

The opinions of commercial rose growers indicate that blindness is caused by the short day length of the winter months. These opinions have not been verified by the results of this experiment. It was found that the decreasing hours of monthly illumination decreased both blind shoots and flower production; whereas an increase in illumination with spring increased flowering and slightly decreased the proportion of blind wood produced. The data indicate that reduced illumination does not stimulate blind shoot production but does have a retarding effect on the total growth of the rose plant.

Growth and differentiation in rose plants varied directly with the quantities of available nitrate in the soil. Low nitrate decreased flower production and increased blind shoot formation; whereas an increase in soil nitrate increased flower production and decreased blind shoot formation. Accompanying the effects of a high nitrate supply on blind shoot formation were the low percentages of reducing sugars found in blind shoots. This relationship between a high nitrate supply and low reducing sugar content and its apparent stimulation on differentiation does not coincide with the

conclusions of other investigators who assert that a low sugar supply accompanied by a high nitrate supply tends to stimulate vegetative growth and promote unfruitfulness.

The chemical analyses showed that flowering shoots contained larger quantities of non-colloidal nitrogen, total nitrogen, insoluble carbohydrates, and total carbohydrates. It is evident that the active forms of carbohydrates are associated with flower production, and the non-colloidal nitrogen and inactive carbohydrates are associated with blindness. There is also a low total nitrogen-total carbohydrate relationship associated with blindness. This relationship between nitrogen and carbohydrate in its relation to blind shoot production is more pronounced in the non-colloidal nitrogen and reducing sugar ratio. In the spring of the year, when flower production is increased, there is an evident increase in the carbohydrate to nitrogen ratios. It is assumed that this increase in ratio brings about a condition which is favorable to flower formation. The increased ratio possibly promotes flowering in shoots which would have otherwise become blind, and because of this the spring increase in flower production is partially accounted for.

Summary

1. A correlation between the physiological behavior and chemical differences of blind and flowering rose shoots indicates that blindness in the rose is a physiological rather than a genetical or a pathological condition.

2. Experiments combining pruning and budding indicate that blindness is a result of the stock and is not due to impotency of the buds. This point is emphasized by the differences in the chemical composition of blind and flowering wood.

3. Growth and differentiation were definitely affected by the monthly hours of illumination and the available nitrate supply, in that a decrease in illumination decreases both flower and blind shoot production while the normal increase in illumination in the spring months increases flower production more rapidly than blind shoot production.

4. With an increase in soil nitrates blind shoot formation decreases and flower production increases. With a decrease in soil nitrates blind shoot formation increases and flower production decreases.

5. The chemical analyses indicate that blindness is associated with high percentages of non-colloidal nitrogen and insoluble carbohydrates; whereas the flowering shoots contain high percentages of reducing sugars.

The writer wishes to express his appreciation to Professors B. S. PICKETT and E. C. VOLZ for their assistance with this problem. Appreciation is also extended to Dr. W. E. LOOMIS for his advice and help in the

performance of the chemical analyses, and for his valuable criticisms on the arrangement and interpretation of the data.

UNIVERSITY OF ARKANSAS
FAYETTEVILLE, ARKANSAS

LITERATURE CITED

1. ANONYMOUS. Roses. Gard. Chron. and Agr. Gaz. **1859**: 28. June 18, 1859.
2. ANONYMOUS. Blindness in roses and pompons. Flor. Rev. **65**: No. 1674. 28. Dec. 26, 1929.
3. CORBETT, L. C. Improvement of roses by bud selection. Mem. Hort. Soc. New York **1**: 93–101. 1902.
4. HOLMES, E. Roses under glass. Flor. Exch. **78**: No. 14. 29. Dec. 5, 1931.
5. LOOMIS, W. E. Methods for the analyses of horticultural material. Proc. Amer. Soc. Hort. Sci. **25**: 59–60. 1926.
6. TAYLOR, W. H. Roses. New Zealand Jour. Agr. **18**: 58. 1919.

RELATION BETWEEN ROOT RESPIRATION AND ABSORPTION

LETA HENDERSON

(WITH TEN FIGURES)

Introduction

Various theories have been developed to explain the nature of the absorption of water by roots, embodying what may be called imbibitional, osmotic, and passive forces (MILLER 9). Imbibitional forces have been emphasized and shown to be of great importance by SHULL (15) and others. Although KUNKEL (7) assumed only imbibitional forces to be effective, more recent investigators agree that osmotic forces must also play an important part. But even these forces fail to explain satisfactorily the transference of water across the endodermis into the vascular cylinder. The nature of the Casparian strip seems to indicate that the transfer of water across this region is under the control of the living cells (PRIESTLEY 12, 13, 14). Also the effect of changing environmental conditions led BLACKMAN (1) to believe that vital relations of the cell, involving the transfer of energy, must be concerned in absorption.

Such energy changes would of necessity be manifested at some time in the respiratory processes of the root. There is no direct evidence showing a correlation between the respirational and absorptive activity of the root except that of NEWTON (10, 11), who found that the evolution of carbon dioxide varied with transpiration and with the osmotic concentration of salt solution supplied in the field. The present study was made to determine more accurately the relation between these processes, using plants under strictly controlled conditions.

In order to reduce individual variations as far as possible, seed from a pure strain of corn (Funk's hybrid no. 517, Yellow Dent) was used in all the experiments. The seeds were placed in water for 12 hours, treated with 4 per cent. formalin for 10 minutes, and washed with sterile water. For germination they were placed under sterile conditions in petri dishes containing filter paper moistened with a few cubic centimeters of culture solution. This treatment resulted in normal and vigorous germination. When the radicles had reached a length of 1–2 cm. the seeds were placed on perforated sheets of cork which were floated in large dishes of Knop's solution. The solution was replaced at intervals of three to five days and continuously aerated. The corn plants were kept in the laboratory at full daylight illumination and grown in the cultures one to two weeks until needed.

Experimentation

The first experiments were carried out by means of the apparatus shown in figure 1. When the plants were 10 inches high they were placed in the experimental chamber (*A*), a 5-inch pyrex test tube with a capacity of

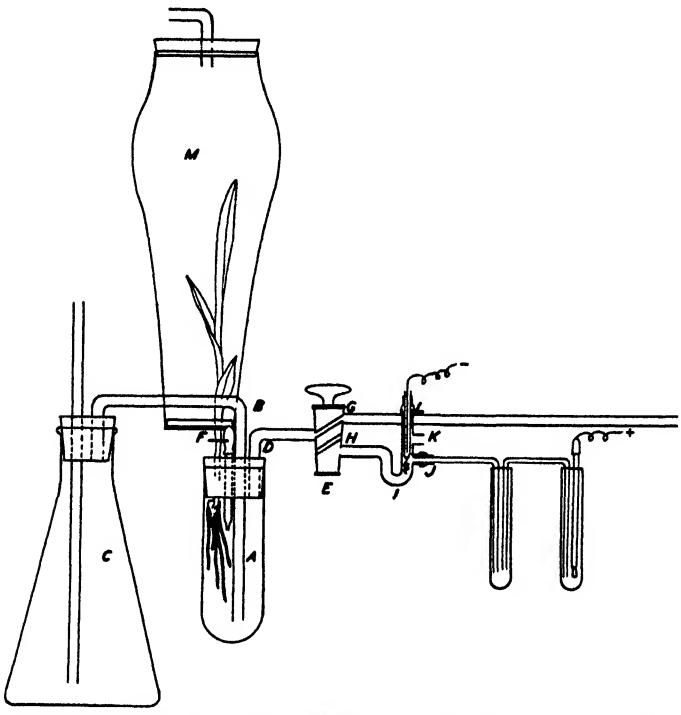


FIG. 1. Diagram of essential parts of apparatus used in determining root respiration by carbon dioxide evolution and water absorption.

90 cc. being used. A rubber stopper was fitted with an inlet tube (*B*) leading from the flask (*C*), which in turn was connected with a large bottle of culture solution. An outlet tube (*D*) led from the test tube to a three-way stopcock (*E*). A section cut from the stopper permitted the plant to be introduced without undue pressure on the stem. A small pipette (*F*) with a rubber bulb was also fitted in the stopper for use in stirring the solution before and after each reading.

The opening (*G*) of the three-way stopcock led to a graduated potometer tube of 1-mm. bore by which was measured the volume absorption during the experimental interval. The second stopcock outlet (*H*) led to the quinhydrone electrode chamber (*I*). Two glass tubes were fused into this chamber; *J* a connection for the KCl bridge, and *K* a waste outlet.

The electrode used was a platinum wire, one end of which was fused into a glass tube with mercury contact with the potentiometer circuit, and the other end coiled to carry crystals of quinhydrone when inserted into the chamber *I*. Short rubber tube connections attached the bridge and the electrode to the chamber and prevented exposure of the solutions to the air during the time of reading.

The plant was inclosed in the chamber *M*, through which air could be circulated, entering under a constant pressure at the top and escaping at the bottom. Moist or dry air could be directed through the chamber, the former passing through three towers containing water and glass wool and the latter through two towers filled with calcium chloride and equipped with dust traps of cotton wool. The apparatus was placed in a thermostat at 25° C. so that only the chamber *M* was subjected to room temperature. Although the room temperature varied from 20° to 25° C. there was seldom more than 2° of variation during a single experiment. Readings were taken at hourly intervals for from 6 to 15 hours.

The rate of transpiration and hence of absorption could be changed by passing either moist or dry air over the plant, and the accompanying effect on root respiration observed. In the first experiments the plants were placed in rain water which had been aerated for 12 hours previously. The plants were transferred from the culture dishes to the observation tubes 10 hours before readings were begun to minimize any effects due to handling. The volume absorption for any period was read directly from the calibrated potometer tube and the evolution of carbon dioxide computed for the same period from the increase in acidity of the solution, since under aerobic conditions carbonic is the only acid excreted by roots (HAAS 5).

After mixing the culture fluid in *A* by means of the pipette *F*, a 10-cc. sample was displaced slowly through the electrode chamber. During the operation the electrode with its load of quinhydrone was elevated above the waste vent *K*. If the displacement is slow and regular, no mixing results in chamber *A*, since the solution in *C* is at the same temperature and is admitted at the end opposite the outlet. The stopcock *E* and the vent *K* are then closed and the electrode slipped down into the liquid. The quinhydrone crystals float slowly down, saturating the liquid in the electrode chamber and allowing a reading to be made. After each reading the culture around the roots was entirely replaced with fresh solution. This was insured by passing 500 cc. of fresh liquid through the culture tube until acidity determinations showed the constant and original value. All distilled water used was freshly made in a block-tin apparatus. Storage vessels used were of pyrex glass.

The carbon dioxide present may be easily determined from the acidity determination. KENDALL (6) has determined the dissociation constant K

of carbonic acid for concentrations up to one atmosphere to be 3.5×10^{-7}; the amount of carbon dioxide may then be determined from (H^+), K, and the volume of the solution used.

$$(1) \qquad (H^+) = \frac{a}{V}$$

where a is the degree of ionization and V is the number of liters which contain one gram mole of carbon dioxide. If K is the ionization constant, the relationship of these two may be expressed:

$$(2) \qquad K = \frac{a^2}{(1-a)V}$$

In these two equations (H^+) and K are known, so the equations may be solved simultaneously and values for a and V determined for a certain pH. From (1) we obtain:

$$(3) \qquad V = \frac{a}{(H^+)}$$

Substituting this value for V in (2) we obtain:

$$K = \frac{a^2}{(1-a)\frac{a}{(H^+)}} \text{ or}$$

$$a = \frac{K}{(H^+) + K}$$

Solving for V in (3)

$$(4) \qquad V = \frac{K}{(H^+)^2 + K(H^+)}$$

Using KENDALL'S value for K, and the (H^+) corresponding to the observed pH, the molarity of the solution may be found from V in this way:

$$C = \frac{1}{V}$$

where C is the number of gram molecules per liter. The actual weight of carbon dioxide in grams per liter may then be calculated. Since the molecular weight of carbon dioxide is 44, the total weight of carbon dioxide present is 44 C. If n represents the number of cubic centimeters of solution used, the weight of carbon dioxide in n cubic centimeters is:

$$(5) \qquad \text{Wt.} = \frac{44Cn}{1000}$$

Since the volume of 1 gram of carbon dioxide at S.T.P. is 509 cc. the volume of gas produced is:

$$(6) \qquad \text{Vol.} = \frac{509 \times 44Cn}{1000} \qquad \text{(at S.T.P.)}$$

Thus it is necessary only to substitute values in equations (4) and (6) to obtain the amount of carbon dioxide.

Results

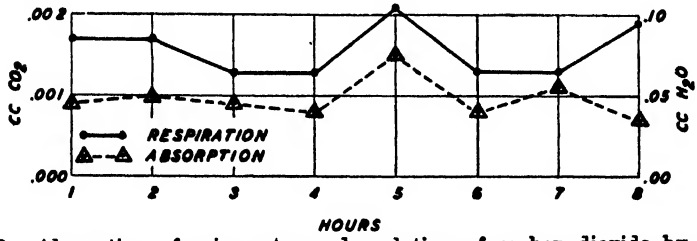


FIG. 2. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 2). Leaves exposed to room conditions.

The results of the first two experiments are shown in table I. Readings were begun at 9 A. M. and the plant tops were exposed to room atmosphere, so that environmental conditions varied throughout the day. Plant 1 showed an unmistakable correlation between absorption and carbon dioxide evolution, although discrepancies appear, particularly toward the end of the experimental period. The results for plant 2 are somewhat more consistent and have been graphed in figure 2. It is evident that this plant showed a remarkably close correlation between respiration and absorption rates, and that there was no departure from this except at the final period

TABLE I

ABSORPTION OF RAIN WATER AND EVOLUTION OF CARBON DIOXIDE BY CORN ROOTS; LEAVES EXPOSED TO ROOM CONDITIONS

HOURS	PLANT 1		PLANT 2	
	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR
	cc.	*cc.*	*cc.*	*cc.*
1	0.050	0.0017	0.040	0.0017
2	0.050	0.0012	0.050	0.0017
3	0.030	0.0012	0.045	0.0013
4	0.020	0.0007	0.040	0.0013
5	0.035	0.0022	0.075	0.0021
6	0.050	0.0022	0.040	0.0013
7	0.070	0.0007	0.055	0.0013
8	0.050	0.0004	0.035	0.0019

of observation. Variable factors evidently influenced respiration and absorption in a similar direction and to a similar degree. Thus the high rate of absorption at the fifth hour is closely paralleled by the rate of respiration, and both rates fell at the sixth hour to lower values. These high rates of absorption and respiration at the fifth hour correspond to the time of maximum illumination in the laboratory.

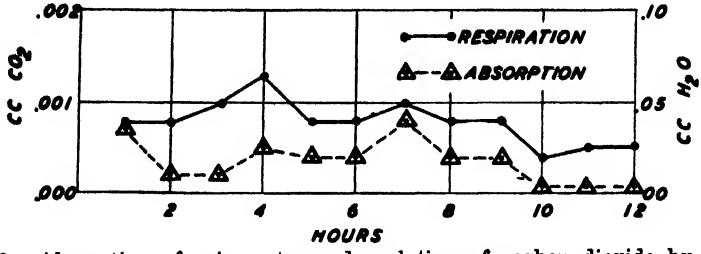


FIG. 3. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 4). Leaves exposed to dry conditions for the initial eight hours.

In table II are shown the results of four experiments carried out similarly to the two previous ones, except that the tops of the plants were inclosed in dark chambers in which the humidity conditions were controlled as indicated. All plants exhibited an essentially similar relation between absorption and respiration. Plant 3 showed considerable variability but a distinct correlation between the two processes. The results for plant 4 are shown in the graph of figure 3. During the initial period of 8 hours the plant was subjected to dry air. During the 12 hours of this experiment the respiration and absorption curves show a close correlation. A high rate of absorption occurred when the plant was first exposed to dry air, but the values quickly fell to a lower level, perhaps indicating the effect of stomatal adjustment. This high initial transpiration rate is not reflected in the rate of carbon dioxide evolution. The change from dry to moist air resulted in a depression of both absorption and respiration rates, a movement which appeared after one hour and required two to reach the minimal level.

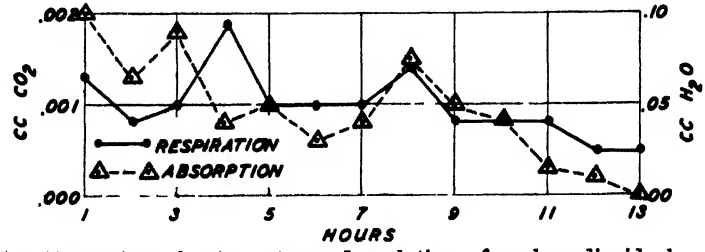


FIG. 4. Absorption of rain water and evolution of carbon dioxide by corn roots (plant 5). Leaves exposed to dry conditions for the initial eight hours.

TABLE II

ABSORPTION OF RAIN WATER AND EVOLUTION OF CARBON DIOXIDE BY CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION

HOURS	PLANT 3		PLANT 4		PLANT 5		PLANT 6	
	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR	ABSORPTION PER HOUR	CO_2 EVOLVED PER HOUR
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.010	0.0067	0.035*	0.0008	0.100*	0.0013	0.025	0.0011
2	0.010	0.0110	0.010*	0.0008	0.065*	0.0008	0.040	0.0007
3	0.015	0.0139	0.010*	0.0010	0.090*	0.0010	0.043	0.0008
4	0.015	0.0139	0.025*	0.0013	0.040*	0.0019	0.040*	0.0006
5	0.060*	0.0132	0.020*	0.0008	0.050*	0.0010	0.075*	0.0008
6	0.040*	0.0132	0.020*	0.0008	0.030*	0.0010	0.075*	0.0007
7	0.065*	0.0160	0.040*	0.0010	0.040*	0.0010	0.040*	0.0005
8	0.070*	0.0160	0.020*	0.0008	0.075*	0.0014	0.040*	0.0005
9	0.055*	0.0160	0.020	0.0008	0.050	0.0008		
10	0.075*	0.0132	0.005	0.0004	0.040	0.0008		
11	0.075*	0.0193	0.005	0.0005	0.015	0.0008		
12			0.005	0.0005	0.010	0.0005		
13					0.000	0.0005		

The graphs of the results obtained for plant 5 are shown in figure 4. The conditions of this experiment were identical with those of the preceding. Here again, although the curves of water absorption and of respiration are variable, they show a good correlation throughout the course of the experiment. During the initial dry period the high rate of absorption of the first hour fell through a variable course to one-third the original value, and then rose. The transfer to moist air was followed, as in the preceding experiment, by a rate of absorption which gradually fell to a minimum. The rate of evolution of carbon dioxide followed the general course of that of absorption, fluctuating during the initial period and gradually falling to a minimum during the exposure to moist air. Similarly, after the initial hour, the two processes recorded for plant 6 showed a close correlation. Since the results obtained from plants 3 and 6 are similar to those of 4 and 5, they have not been graphed.

Because the respiratory values obtained in these experiments seemed low, it was thought that buffer substances were present in the rain water, preventing a hydrogen ion concentration corresponding to the total amount of carbonic acid present. Freshly distilled and aerated water was therefore used in place of rain water. No toxic effects could be detected even over periods much longer than the duration of the experiments. The higher values obtained seemed to confirm the assumption just mentioned. Thus the values obtained in rain and distilled water cannot be compared, but only the correlation between root respiration and absorption in the two media.

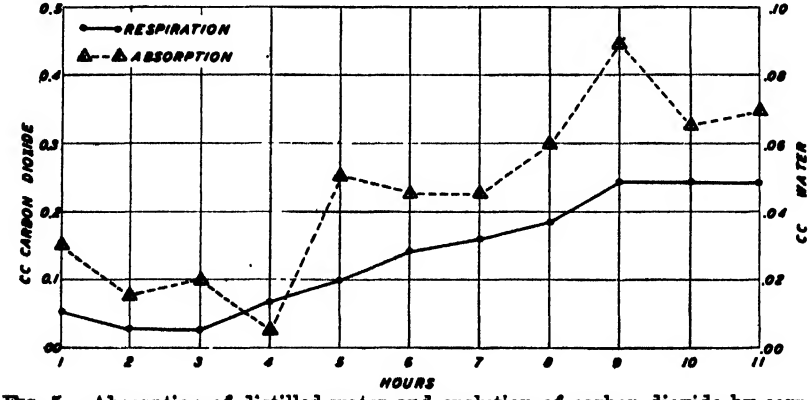


FIG. 5. Absorption of distilled water and evolution of carbon dioxide by corn roots (plant 7). Leaves exposed to moist air for the first four hours.

The results obtained when distilled water was used are shown in table III for plant 7 which was kept in a saturated atmosphere for the first four hours of the experimental period. The curves in figure 5 show a good

TABLE III

ABSORPTION OF DISTILLED WATER AND RESPIRATION OF CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION. RESPIRATION MEASURED IN PLANT 7 BY EVOLUTION OF CARBON DIOXIDE, IN 9 BY CONSUMPTION OF OXYGEN, AND IN 8 BY BOTH PHENOMENA

Hours	Plant 7		Plant 8				Plant 9		Average for 8 and 9	
	Absorption per hour	CO_2 evolution per hour	Absorption per hour	CO_2 evolution per hour	O_2 consumption per hour	R.Q.	Absorption per hour	O_2 consumption per hour	Absorption per hour	O_2 consumption per hour
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	0.030	0.0532	0.045	0.1909	0.212	0.91	0.040*	0.085	0.043	0.149
2	0.015	0.0285	0.020	0.0714	0.085	0.84	0.070*	0.127	0.045	0.106
3	0.020	0.0258	0.030*	0.0777	0.127	0.61	0.080*	0.169	0.055	0.148
4	0.005	0.0692	0.035*	0.1034	0.169	0.61	0.090*	0.127	0.063	0.148
5	0.050*	0.1099	0.035*	0.1034	0.169	0.61	0.112*	0.169	0.074	0.169
6	0.045*	0.1438	0.050*	0.1034	0.169	0.61	0.070*	0.085	0.060	0.127
7	0.045*	0.1623	0.060*	0.1034	0.169	0.61				
8	0.060*	0.1873	0.060*	0.1096	0.169	0.64				
9	0.090*	0.2456	0.030*	0.1096	0.169	0.64				
10	0.065*	0.2431								
11	0.070*	0.2431								

correlation between the rates of respiration and absorption. Both rates remained at low levels during the initial four-hour moist period and rose to maximum values at the ninth hour. In this unbuffered solution the values obtained probably more nearly represent the respiratory activity of the root system than do those for the plants observed in rain water. It is significant that in both rain and distilled water there is a good correlation between the respiratory and the absorptive activities of the root system.

For following the respirational activity, the micro-Winkler method of measuring dissolved oxygen was employed either alone or with the electrometric method of carbon dioxide estimation. As used by many workers, this modification of the original Winkler method requires a sample of but 10 cc. To obtain greater accuracy the sodium thiosulphate solution was used in a concentration of N/160 instead of the usual N/40, and was added from a micro-burette. A Thompson and Miller apparatus as described by ZIMMERMAN (**16**) was found best suited to the work. The sample for analysis was withdrawn, with proper precautions against agitation and exposure to the air, through the potometer tube into the apparatus. The reagents were added immediately from the attached burette tubes and titration promptly carried out.

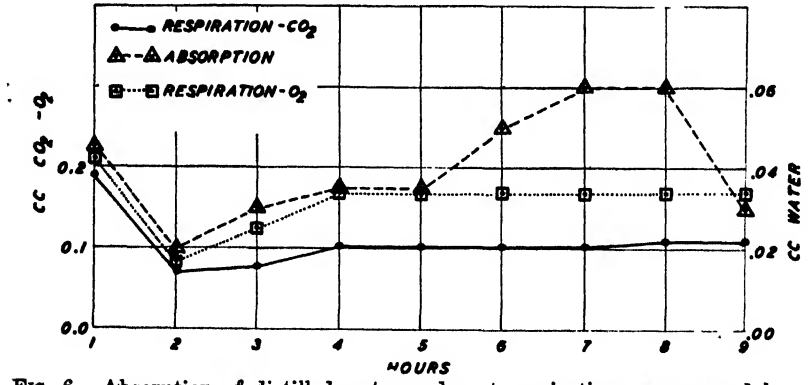


FIG. 6. Absorption of distilled water and root respiration as measured by carbon dioxide evolution and oxygen consumption (plant 8). Leaves exposed to moist conditions for the initial two hours.

The respiration of plant 8 was measured by the oxygen consumption and the carbon dioxide evolution while the root system was immersed in aerated distilled water. The results are given in table III and the curves shown in figure 6. The plant was exposed to moist air for two hours and to dry for the remainder of the experimental period. The curves show a general correlation, the discrepancy being the high rates of the sixth to eighth hours. From the initial high reading all curves fell to minimum values during the second hour, and, except for the high values just men-

tioned, rose to a moderate level for the remainder of the experiment. The curves of carbon dioxide evolution and of oxygen consumption parallel each other throughout the course of the experiment. It is evident that the rate of root respiration measured by either of the two methods gives curves which differ only in level.

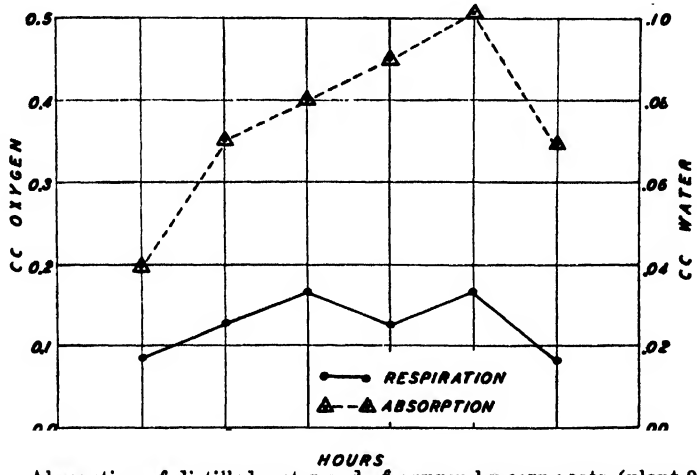


FIG. 7. Absorption of distilled water and of oxygen by corn roots (plant 9). Leaves exposed to dry conditions during the entire experiment.

The root respiration of plant 9 was followed by means of the micro-Winkler method while kept in aerated distilled water. The results are given in table III and the curves shown in figure 7. Oxygen and water absorption show a general correlation. During the initial three hours both

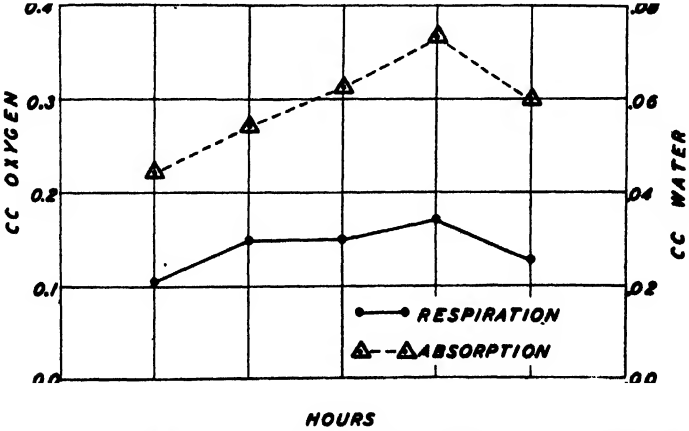


FIG. 8. General course of root respiration and absorption in distilled water (averages of plants 8 and 9).

rates increased, a movement which continued through the following two hours only for the absorption of water. Both curves rose to maxima at the fifth hour and fell to lower values at the sixth. These variations may have been due to changes in the rate of air flow over the tops, or to periodic fluctuations in the plant itself.

The average values from plants 8 and 9, showing the absorption of distilled water and of oxygen, are given in table III and the curves of the values are shown in figure 8. The first hour, during which the plants were not adjusted to the experimental condition, has not been included. The correlation between the two processes is evident.

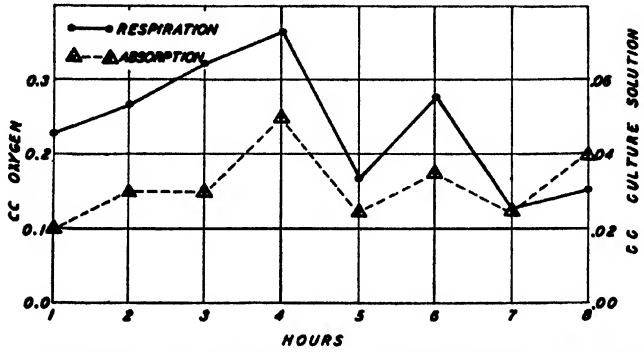


FIG. 9. Absorption of culture solution and oxygen consumption by corn roots (plant 11). Leaves surrounded by moist air for the initial hour.

Since the rain water and distilled water used in the experiments might be thought to produce abnormal root behavior, further work was carried out using culture solutions similar to those in which the plants had been grown. It is obvious that buffer action does not interfere with the determination of oxygen, and that the micro-Winkler method can be used with equal accuracy for observations in distilled water, rain water, or mineral nutrient solutions. Experiments 10, 11, and 12 were carried out in Knop's solution identical in composition with that in which the plants had been grown. The results are given in table IV. The curves of the values obtained for plant 11 are shown in figure 9 as typical of the set. It will be seen that a close correlation between respiration and absorption was shown, the two processes paralleling each other throughout the course of the experiment. From the initial low values obtained under conditions of moist air, the maximum rates of both respiration and absorption were reached at the fourth hour, after which the two processes varied simultaneously and similarly. The results obtained for plants 10 and 12 are similar to those for plant 11. The effects of moist and dry air are not so obvious as in earlier experiments.

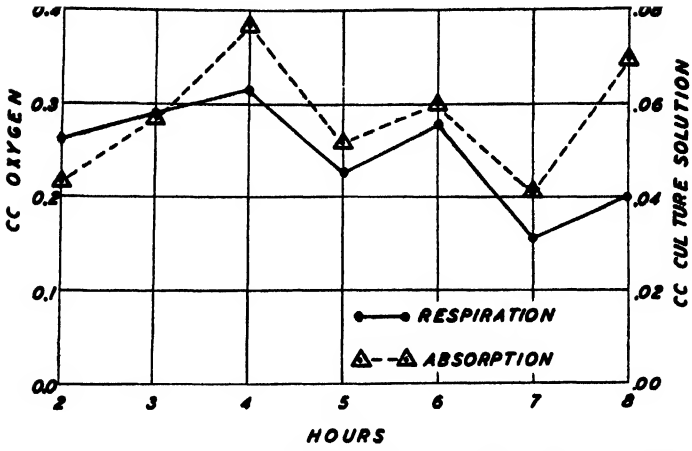


FIG. 10. General course of root respiration and absorption in culture solution. Averages of plants 10, 11, 12.

The averages for the absorption of water from the culture solution and for the consumption of oxygen are given in table IV and the curves of these values are shown in figure 10. At the first reading in experiment 12 the plant apparently had not become adjusted to the experimental conditions and hence this value is not shown in figure 10. It will be seen that the correlation between the absorption of liquid and of oxygen is close, the processes varying in parallel throughout the period.

Discussion

The thermodynamic conception of an organism is less prominent in the work of the plant physiologist than in that of workers in the field of animal physiology. Respiration, as an indication of physiological activity, may be taken as a measure of work done, even when the exact nature of this work is not evident. The thermodynamic relations of plant activities have received too little attention. Comparatively few detailed studies of the energy relations of any plant activity have been reported. Thus while we know how to evaluate respiration in terms of energy, we do not possess such knowledge concerning the use of this energy in the plant. For instance, in the fundamentally important water relations of plants many workers have hesitated to assign any endothermic physiological relation. Since the work of DUTROCHET, the osmotic mechanism is a widely accepted explanation of water absorption and transport, in spite of the fact that many of the concentration gradients along the paths do not agree in direction with that of the movement of water.

Following the work of SACHS, imbibitional forces have been accepted by

TABLE IV

ABSORPTION OF CULTURE SOLUTION AND OF OXYGEN BY CORN ROOTS; TOPS INCLOSED IN DARK CHAMBERS WITH MOIST OR DRY (*) AIR CIRCULATION

HOURS	PLANT 10		PLANT 11		PLANT 12		AVERAGE	
	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR	ABSORPTION PER HOUR	O_2 CONSUMPTION PER HOUR
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.020	0.215	0.020	0.229	0.050	0.449	0.030	0.298
2	0.050*	0.355	0.030*	0.269	0.050	0.169	0.043	0.264
3	0.090*	0.373	0.030*	0.322	0.050	0.178	0.057	0.291
4	0.110*	0.338	0.050*	0.364	0.070*	0.237	0.077	0.313
5	0.060*	0.279	0.025*	0.169	0.070*	0.237	0.052	0.228
6	0.075*	0.322	0.035*	0.279	0.070*	0.237	0.060	0.279
7	0.060*	0.245	0.025*	0.127	0.040*	0.102	0.042	0.158
8	0.120*	0.322	0.040*	0.152	0.050*	0.127	0.070	0.200

many workers as an explanation of water absorption. KUNKEL (7) believed these forces to be the main ones concerned in the intake of water by roots, and that osmotic forces play no part. SHULL (15) assigned an important rôle to imbibitional forces, but did not exclude the osmotic. According to either of these conceptions, the energy for the absorption of water would be the absorbed radiant energy necessary for vaporization and for photosynthesis in the aerial parts of the plant. Both the imbibitional and the osmotic explanations of water absorption and transport fail to explain satisfactorily exudation pressure and also the fact that an oxygen supply is necessary for normal root activity. Similarly guttation is explained with difficulty on this basis. In order to explain the transport of water under these conditions various hypotheses have been suggested. PRIESTLEY and NORTH (14) believed the endodermis to be the functional absorbing surface of the root; they assign to it the rôle of a semipermeable membrane, separating the intercellular cortical fluids from those of the xylem vessels. PFEFFER formulated a hypothesis based on differences in the osmotic pressure of the plasma membrane in different parts of the cell to explain the movement of water from a solution of high concentration to a low one. Although he later abandoned this as being unlikely, LEPESCHKIN (8) used it to explain the exudation of water by sporangiophores and by hydathodes.

More recently workers coming from the field of animal to general physiology have considered that osmotic work must be done. The actual mechanism involved is not yet clear. A secretory action may take place in the endodermis or in other tissues; but since the mechanism of glandular activity is still imperfectly understood, such a hypothesis is not very clarifying. Similarly electro-endosmosis is a possible explanation, but until more facts substantiating this have been found such an assumption is of little value.

It is apparent that irrespective of any mechanism involved, osmotic work must be done. Any system of cells along the line of water absorption and transport must maintain a gradient of increasing osmotic pressure from root hair to leaves. This will be seen to be true whether the osmotic or the imbibitional mechanism be assumed, since in any active cell there will be an equilibrium between the forces of the colloids present in the cell walls and the osmotic activities of the solutes of the cell sap. Since the actual concentration gradient observed does not conform to this requirement, and since positive exudation pressures are not thus explicable, these theories are incomplete.

If work is accomplished, the necessary energy must be furnished directly or indirectly by respiration processes and can be detected and estimated by them. The present study was undertaken with this in view.

The results obtained from a simultaneous measurement of water absorption and root respiration, as measured by the evolution of carbon dioxide

computed from the changes of pH in rain water, show a close correlation between the two processes. Even in the two experiments where the plants were exposed to light this relation was evident. When transpiration was increased by changing the air over the tops from a high to a low humidity, the rate of absorption increased and was accompanied or closely followed by a rise in the rate of carbon dioxide evolution.

When the evolution of carbon dioxide was followed in aerated distilled water a correlation similar to that observed in rain water appeared. In general this correlation was somewhat more perfect than that observed in rain water.

It is to be noted that, assuming the most intimate relation between root respiration and the absorption of water, a perfect correspondence between the rates would not be expected in experiments such as those here reported. This arises from the fact that some of the respiratory carbon dioxide is carried up in the transpiration stream and hence is not evolved (CERIGHELLI **2, 3, 4**). This may be considered as the explanation for the low rate of respiration accompanying high absorption at such points as the third hour of figure 4; and also for the failure, in cases like that shown in figure 6, of the respirational curve to follow the rise of absorption. Since the curves of carbon dioxide evolution and of oxygen consumption are parallel, any lack of agreement between the former phenomenon and the absorption of water is without significance in these experiments. This study gives no evidence of the transport of carbon dioxide or of any effects upon the evolution of the substance due to the photosynthetic activity of the leaves.

Computations from the results of experiments 1–6, in which the root systems were observed in rain water, show great variability and an average of 0.08 cc. (S.T.P.) carbon dioxide evolved per cubic centimeter of water absorbed. A similar average for plants observed with the root systems in distilled water is 2.6 cc. This difference is doubtless due to the buffering substances present in rain water and the latter value is to be regarded as the more accurate.

The consumption of oxygen offers a more reliable measurement of respirational activity, and in experiment 8 the absorption of 1 cc. of water required 3.9 cc. of oxygen and evolved 2.6 cc. of carbon dioxide, giving a respiratory quotient of 0.67. The average consumption of oxygen per cubic centimeter of water absorbed from distilled water is 2.4 cc. If we accept the value of 5.047 small calories per cubic centimeter of carbon dioxide, we have in experiment 8 an expenditure of approximately 13.1 calories per cubic centimeter absorption. If the consumption of oxygen be considered a more accurate measure of work, the result is approximately 19.7 calories.

Similar computations for plants observed with the root systems in Knop's solution give an oxygen consumption of 4.7 cc. per cubic centimeter

absorption. The average consumption of oxygen per volume absorption in distilled water is but 52 per cent. of that in Knop's solution. Computed on the basis of the average values obtained in distilled water, the oxygen consumption of the root systems in Knop's solution is 193 per cent. Comparing the calorific values per cubic centimeter absorption, we have 13 calories for distilled water and 23.7 for Knop's solution. Since the osmotic pressure of the Knop's solution used was 1.75 atmospheres, it appears that the energy required for the absorption of distilled water is equivalent to that expended against an osmotic pressure of $\frac{23.7 - 13}{1.75 \times 13}$, or 2.12 atmospheres.

In view of the fact that the resistance to flow through capillary tubes is a linear function of the rate, it might be expected that the energy requirement would vary directly with the rate of absorption. Computations show that the reverse relation holds in the present study, doubtless because the basal rate for the tissues comprises an increasing proportion of the whole, since at the lower absorption rates the time interval is increased.

It is to be noted that the respiration as here recorded was not anaerobic or modified by deficient aeration. This is obvious from the fact that the fluid around the roots was replaced at hourly intervals with aerated medium, and that the amount of dissolved oxygen contained in this was 1.4 cc. The observed absorption was never more than 0.5 cc. and usually was much less than this amount.

For corn seedlings in liquid cultures the correlation between the volume absorption and the respiration of the root indicates the expenditure of energy by these organs in the process of absorption. Further and more exact determinations of the energy values with plants under strictly controlled conditions should throw much light on the nature of the mechanism involved.

Summary

1. A method was developed for obtaining simultaneous measurements of root respiration and volume absorption of the roots.

2. The evolution of carbon dioxide was measured by change in the pH of the medium in the absence of buffers, and the absorption of oxygen was measured by the micro-Winkler method.

3. A correlation between the evolution of carbon dioxide, the absorption of oxygen, and the volume absorption of the root system of corn seedlings is shown to occur in rain water, distilled water, and in Knop's solution.

4. The energy as calculated from the respiratory exchange is 93 per cent. greater in Knop's solution with an osmotic pressure of 1.75 atmospheres than in water.

5. The absorption of water by corn roots is accompanied by the expenditure of energy.

The writer wishes to thank Dr. G. W. GOLDSMITH and others associated in the physiological work at the University of Texas for constructive suggestions and criticism.

UNIVERSITY OF TEXAS
AUSTIN, TEXAS

LITERATURE CITED

1. BLACKMAN, V. H. Osmotic pressure, root pressure and exudation. New Phytol. **20**: 106–115. 1921.
2. CERIGHELLI, RAOUL. Sur les échanges gazeux de la racine avec l'atmosphere. Compt. Rend. Acad. Sci. (Paris) **171**: 575–578. 1920.
3. ———. Nouvelles recherches sur la respiration de la racine. Rev. Gén. Bot. **37**: 102–112. 1925.
4. ———. Nouvelles recherches sur la respiration de la racine. Rev. Gén. Bot. **37**: 157–166. 1925.
5. HAAS, A. R. C. The excretion of roots. Proc. Nat. Acad. Sci. **2**: 561–566. 1916.
6. KENDALL, JAMES. The specific conductivity of pure water in equilibrium with atmospheric carbon dioxide. Jour. Amer. Chem. Soc. **38**: 1480–1497. 1916.
7. KUNKEL, L. O. A study of the problem of water absorption. Missouri Bot. Garden Ann. Rept. **23**: 26–40. 1912.
8. LEPESCHKIN, W. W. Zur Kenntnis der Mechanismus der aktiven Wasserausscheidung der Pflanzen. Beih. z. bot. Centralb. Abt. 1. **19**: 409–452. 1906.
9. MILLER, EDWIN C. Plant physiology. McGraw-Hill, New York. 1931.
10. NEWTON, J. R. Measurements of carbon dioxide evolved from roots of various crop plants. Sci. Agr. **4**: 268–274. 1924.
11. ———. The relation of the salt concentration of the culture solution to transpiration and root respiration. Sci. Agr. **5**: 316–320. 1925.
12. PRIESTLEY, J. H. The mechanism of root pressure. New Phytol. **19**: 189–200. 1920.
13. ———. Further observations upon the mechanism of root pressure. New Phytol. **21**: 41–48. 1922.
14. ———, and NORTH, E. E. Physiological studies in plant anatomy. III. The structure of the endodermis in relation to its function. New Phytol. **21**: 113–139. 1922.
15. SHULL, C. A. Imbibition in relation to absorption and transportation of water in plants. Ecology **5**: 230–241. 1924.
16. ZIMMERMAN, P. W. Oxygen requirements for root growth of cuttings in water. Amer. Jour. Bot. **17**: 842–861. 1930.

CARBOHYDRATE–NITROGEN AND BASE ELEMENT RELATIONSHIPS OF PEAS GROWN IN WATER CULTURE UNDER VARIOUS LIGHT EXPOSURES

ORMAN E. STREET

(WITH THREE FIGURES)

Introduction

The response of plants to the supply of various nutrient elements has been the subject of numerous investigations. The degree of response is largely governed by environmental factors, which may even nullify the effect of varying treatments. A dynamic material such as soil is apt to be much less responsive to treatment than is quartz sand, which in turn is a less responsive medium than are water cultures. It is often possible to obtain significant differences in growth and composition of plants in water cultures when the differences in nutrient supply are rather small.

In studies of carbohydrate-nitrogen relationships, the tendency has been to employ radical treatments, especially as regards the nitrogen supply, conclusions being advanced on comparisons between a very low and a very much higher level of nitrate nitrogen in the culture medium. In the present work, the nitrogen supply has been kept at the same order of magnitude in all cultures. The employment of differential water cultures of the same total osmotic concentration permitted this control.

Considerable differences in the organic composition of peas grown in water cultures were reported in 1928 by CLEMENTS (7). He concluded that under long light exposures nitrogen assimilation was influenced by the supply of potassium, and total carbohydrates were highest with high nitrogen treatments, while under shorter light exposures the greatest nitrogen assimilation was with an intermediate treatment, and the highest total carbohydrates with low nitrogen supply. The present work was started in the same laboratory with the purpose of testing these conclusions.

In view of the well established effect of light duration on growth and reproduction, the use of several light exposures was a necessary part of this study. As a continuation of this phase of the problem, it was decided to investigate the effect of light duration on the intake of base elements by the plant. Such problems as the effect of the season on the composition of the plant are perhaps related to light duration.

Literature review

The extensive literature on water cultures has been thoroughly reviewed by TOTTINGHAM (**25**) and others, and need not be enlarged here. The work of SHIVE (**24**) on salt combinations and of MCHARGUE (**19**), BRENCHLEY

(6), MAZÉ (20), and others on supplementary elements is also well reviewed. The exhaustive studies of GARNER and ALLARD (9, 10, 11) on light effects in plants have been extended by numerous workers, and the literature is well summarized by ARTHUR, GUTHRIE, and NEWELL (3). These investigators also attempted to control other climatic factors, such as moisture, temperature, and carbon dioxide concentration of the air, as well as the intensity, quality, and duration of light. All the studies support the generalization that an extension of light period brings about an increase in carbohydrate content and a decrease in nitrogen content.

The intake of base elements is apt to be a function of other factors than the supply of any one element, but under optimum conditions an intake proportionate to the supply will obtain. Among the fertilizer elements that cause a marked response in the plant, potassium is usually foremost. ANDERSON, SWANBACK, and STREET (2) reported a range of 1.16 to 6.78 per cent. K_2O on tobacco grown under various conditions. FONDER (8) has noted consistent differences in potash content of alfalfa on several soil types, while SAYRE (23), working with canning peas, obtained a range from 0.87 to 8.70 per cent. by varying the CaO/K_2O ratio in water cultures.

Responses to calcium and magnesium applications in soil are usually related to the relative abundance of these elements as compared with the potash supply, as has been shown by MORGAN (21) and HAAS (12). Application of lime materials containing both calcium and magnesium results in an increase of the magnesium content, but notably depresses the potassium and strongly reduces the calcium content as indicated by the work of ANDERSON *et al.* (2). The studies of MORGAN (21) also show that it is only when both potassium and magnesium are available in relatively small amounts that the plant is able to take up calcium freely.

References to the effect of light on the intake of bases are not numerous in the literature. BARTHOLOMEW and JANNSEN (5) conclude that potassium is taken up as freely at night as during the day. TYSON (26) grew sugar beets in compartments shaded to varying degrees, and found that the crude ash content of leaves increased with a decrease of light intensity, and that in general the same relation held for the three principal bases. NIGHTINGALE *et al.* (22) found that plants grown in a calcium deficient medium, when placed in darkness, showed the presence of "uncombined" calcium, *i.e.*, calcium that could be detected by the usual treatment with oxalic acid, and that the presence of calcium in this form permitted growth and perhaps protein elaboration.

Plan of experiment

The triangular system of TOTTINGHAM (25) and SHIVE (24), in which three-salt combinations having a total osmotic concentration of one atmos-

phere are used, was selected as a culture medium for these experiments. The same salt combination (KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$) as was used by CLEMENTS (7) was employed. Three-gallon crocks fitted with paraffined aluminum covers and capable of growing 40 plants were used, and duplicate cultures of each treatment grown. The cultures listed in table I were chosen as being representative of different portions of the triangle, as well as giving several combinations of potassium and nitrogen, under the conditions recommended by LIVINGSTON *et al.* (17).

TABLE I

DIFFERENTIAL CULTURE SOLUTIONS, TYPE 1
OSMOTIC CONCENTRATION, 1 ATMOSPHERE

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME MOLECULAR PROPORTIONS		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1–1–6	1	1	6	0.0027	0.0027	0.0161
1–6–1	1	6	1	0.0020	0.0122	0.0020
2–2–4	2	2	4	0.0049	0.0049	0.0099
3–3–2	3	3	2	0.0068	0.0068	0.0045
5–1–2	5	1	2	0.0123	0.0024	0.0049

In addition to the salts employed (table I), small amounts of iron, manganese, and boron were added to the culture solutions. Preliminary tests showed that 2.3–4.0 p.p.m. iron in ferric tartrate, 0.66–2.0 p.p.m. boron in boric acid, and 0.55–2.0 p.p.m. manganese in manganous chloride were the optima, and the lower amounts were used in subsequent experiments.

The culture solutions were changed once a week. Transpiration losses were made up by the addition of distilled water during the latter part of the growing period. The cultures were aerated for a period of two hours each day.

Light exposures of 10, 13, and 17 hours were chosen as representing the range of light period under which normal growth might be expected. The longer periods were in part artificial light, the ten cultures of each group being arranged under a battery of lights suspended 5 feet above the greenhouse benches and supplying 2000 watt at 110 volts. Measurements of light intensity were not made, as the duration of light has been found by GARNER *et al.* (11) to be the most significant factor, provided the intensity is not too low. However, comparison of illumination per unit area (as used in these experiments) with that employed by ARTHUR *et al.* (3) would indicate that the intensity was high. No water screens between the lights

and the plants were available but the heating effect was not noticeable. As the plants grew taller, the lights were raised.

The 10-hour plants received only solar light, altered, of course, by the glass of the greenhouse. These cultures were grown in large wooden boxes which were fitted with light-tight covers and ventilated by a shutter arrangement similar to that employed in photographic dark rooms. The period of illumination of these cultures was 8 A. M. to 6 P. M.

The 13-hour plants were protected from the artificial light source of the 17-hour plants by curtains of fine black cambric cloth. These curtains were drawn around the bench at the end of the 13-hour period and withdrawn early in the morning.

Nott's Excelsior field peas, obtained from the Michigan State Farm Bureau, Lansing, Michigan, were used throughout the experiments. They were found to be an excellent type for this work, having a sturdy growth habit and flowering freely. Before being placed in the germinator the seeds were sterilized in 1–250 formalin for 20 minutes, washed in tap water, and soaked for a few hours. They were then placed between layers of moist paper toweling in a large galvanized iron pan. After about three days a uniform lot of the more vigorous seedlings with roots measuring one inch in length was selected for the experiment. These selected seedlings were fitted into perforated corks each of which held five plants. The three-gallon culture jars were fitted with perforated aluminum covers, each of which held eight corks, making a total of 40 plants per culture.

In the first two series the seedlings which were damaged in handling were replaced during the first few days of the experiment. In the final series several extra corks were prepared and allowed to remain in contact with tap water until the experiment had progressed about two days, when replacement of corks not having a complete stand was made. By the latter technique a somewhat more uniform stand was obtained.

The chemical analyses were made on the tops and pods only, as follows: nitrate nitrogen, total nitrogen not including nitrates, simple sugars, sucrose, starch and hemicellulose, crude ash, potassium, calcium, and magnesium. Physical measurements included top length in centimeters, green weight of tops (and pods when found), oven-dry weight of tops, roots, and pods.

Chemical methods

In order to obtain comparable results, the chemical methods for carbohydrates and total nitrogen not including nitrates (organic nitrogen) were the same as those reported by CLEMENTS (7). All samples for the determination of carbohydrates were preserved in alcohol to which a small quantity of ammonium hydroxide was added to neutralize plant acids.

The DEVARDA method for nitrate nitrogen was tried but gave such

unreliable results that it was soon discarded. The impossibility of preventing breakdown of the simpler nitrogenous compounds such as amino acids and amides, and the consequent high results, render the method worthless for plant material. The modification of the GILBERT method by HOLTZ and LARSON (16) gave very good results.

Crude ash and calcium were determined by the official methods of the A.O.A.C. (4). Magnesium was determined by the volumetric method of HANDY (13). Potassium determinations of the first series were made by the sodium cobalti-nitrite method of ADIE and WOOD (1). A comparison of this method with the official chloroplatinate procedure showed that the former method did not give consistent results, most of the percentages being too high. Series I was therefore repeated and all potassium figures reported are by the official method. All figures reported are on oven-dry-weight basis.

Experimental results

Series I was started March 13 and ran until May 14, 1928, a period of nine weeks. Blossoms appeared under 17-hour light three weeks after transplanting the seedlings. Both of the longer light periods produced pods abundantly, and at the time of harvest the pods were beginning to ripen. The short-light plants blossomed toward the end of the period, but developed very few pods. Control of the temperature in the greenhouse was not difficult during these months, and cultural conditions were generally satisfactory. It was necessary to use lemon oil spray to control red spiders in the greenhouse and to give the cyanide treatment for white flies and aphids. The plants were not at any time seriously affected by these insects.

Series II ran from June 13 to July 31, 1928, a period of seven weeks. Owing to the higher air temperature in the greenhouse at this time, the plants matured more rapidly, and the uniformity of plants in a single culture was not so satisfactory. The light intensity was also higher at this later time, and this apparently had some effect on the growth in height and the carbohydrate content of the plants.

In series III it was decided to limit the experiment to 10-hour and 13-hour light periods, to have treatments in triplicate, and to terminate the experiment when the plants were vegetatively developed but had not set fruit. The cultures were started May 14 and ran until June 12, 1929, a period of four weeks. At this season of the year the days were long enough to omit the use of artificial light for any part of the 13-hour period.

PHYSICAL MEASUREMENTS

Physical measurements for all series are presented in table II. In order to establish the relative value of the various treatments in dry weight

production, the total dry weight per plant, exclusive of pods, was reduced to a ratio, taking the best treatment in each group as unity. These ratios are given in the last column of the table.

The average top length was usually greatest in the treatments supplying the highest proportion of nitrates. The dry weight production was highest under similar conditions, solution 1–6–1 leading in six out of eight groups and solution 3–3–2 being second best in the same number of cases. A weighted average of these ratios shows that the latter solution was 98.9 per cent. as efficient as the former in dry weight production. Solution 5–1–2, high in potassium, produced 86.9 per cent. of the dry weight of the 1–6–1 treatment; while 1–1–6 and 2–2–4, both high in magnesium, each produced 83.2 per cent.

Carbohydrate-nitrogen relationships

Results of carbohydrate analysis of series I, tops, are shown in table III. The term tops as used in this paper includes all the aerial parts of the plant except pods. It was not possible to separate these parts and secure samples large enough for analysis. All carbohydrates are computed to d-glucose using Munson and Walker's table (4). The figures presented are the average of the duplicate cultures, with duplicate determinations of each sample. Thus each entry represents the average analysis of a group of 80 plants.

As an aid to interpretation of the results, the ratio system was again employed. Ratios were computed upon the total sugars (sum of the simple sugars and sucrose), total non-sugars (sum of starch and hemicellulose), and total carbohydrates.

In this series the culture solutions highest in magnesium sulphate were the most efficient in promoting the elaboration of sugars. This was true in all light exposures, the only discrepancy being in the 2–2–4 treatment in the 13-hour light period. Solution 5–1–2, high in potassium, was not quite so efficient, while the high nitrate solution was only 70 per cent. as high in total sugars as the 1–1–6 treatment.

In the formation of more stable carbohydrate compounds, the most efficient solution was found to be the high potassium treatment. Although it did not consistently lead in all light exposures, the average of the ratios placed it in the fore. The magnesium treatments were consistently good, while the high nitrate treatment was again the least efficient.

In a study of the total carbohydrates, the most striking difference is the low total content of the plants in solution 1–6–1. Because of the superiority of the magnesium treatments in the production of sugars and the potassium treatments in the transformation of sugars into non-sugars, the two groups are about equally effective in maintaining a high total carbohydrate level.

It is apparent from the data that the duration of light was a significant factor in determining the general level of carbohydrate storage in the plant.

Table III also presents the results of determinations of organic and

TABLE II

PHYSICAL MEASUREMENTS OF PEAS GROWN IN WATER CULTURE

TREATMENT*	AVERAGE TOP LENGTH	AVERAGE DRY WEIGHT PER PLANT			DRY WEIGHT† (RATIO)
		TOPS	ROOTS	PODS	
	SERIES I				
	17 hours light				
	cm.	*gm.*	*gm.*	*gm.*	
1–1–6	52.5	0.8363	0.1860	0.6390	0.80
1–6–1	52.5	1.0865	0.2774	0.7719	1.00
5–1–2	47.0	0.7649	0.1904	0.5500	0.70
3–3–2	51.6	0.9184	0.2755	0.6210	0.87
2–2–4	45.5	0.6216	0.1559	0.5206	0.57
	13 hours light				
1–1–6	50.8	0.7886	0.1535	0.4051	0.70
1–6–1	50.6	0.8404	0.1817	0.3264	0.76
5–1–2	49.9	0.9025	0.2349	0.3704	0.85
3–3–2	53.9	0.9300	0.3826	0.4315	0.98
2–2–4	50.8	1.0812	0.2527	0.5277	1.00
	10 hours light				
1–1–6	39.0	0.4537	0.1058		0.67
1–6–1	45.6	0.7007	0.1361		1.00
5–1–2	33.5	0.4195	0.1066		0.62
3–3–2	40.8	0.4984	0.1429		0.76
2–2–4	41.5	0.4402	0.1506		0.71
	SERIES II				
	17 hours light				
1–1–6	36.5	0.6640	0.0962	0.1854	0.71
1–6–1	38.8	0.9160	0.1456	0.2703	1.00
5–1–2	36.3	0.7541	0.1328	0.2105	0.83
3–3–2	39.7	0.8785	0.1297	0.1171	0.94
2–2–4	34.4	0.7158	0.0979	0.1248	0.76
	13 hours light				
1–1–6	33.8	0.5183	0.0824	0.0578	0.75
1–6–1	42.0	0.7092	0.0896	0.1000	1.00
5–1–2	39.0	0.6612	0.1152	0.0567	0.97
3–3–2	36.9	0.6142	0.0998	0.1153	0.89
2–2–4	31.0	0.4919	0.0792	0.0828	0.77
	10 hours light				
1–1–6	21.8	0.2759	0.0337		0.81
1–6–1	24.2	0.2296	0.0400		0.70
5–1–2	24.3	0.2604	0.0527		0.79
3–3–2	25.3	0.3313	0.0624		1.00
2–2–4	23.5	0.2233	0.0467		0.68

TABLE II-

Treatment*	Average top length	Average dry weight per plant			Dry weight† (ratio)
		Tops	Roots	Pods	
	Series III *13 hours light*				
1–1–6	24.6	0.3386	0.0830		0.89
1–6–1	32.4	0.3832	0.1012		1.00
5–1–2	30.0	0.3275	0.0936		0.86
3–3–2	30.6	0.3526	0.0939		0.92
2–2–4	25.4	0.3327	0.0785		0.84
	10 hours light				
1–1–6	24.3	0.2609	0.0604		0.92
1–6–1	25.7	0.2758	0.0722		1.00
5–1–2	20.3	0.2366	0.0624		0.86
3–3–2	22.8	0.2760	0.0657		0.98
2–2–4	24.7	0.2500	0.0614		0.89

* Culture solution, Shive's 1.00 atmosphere: 1st figure $KH_2(PO_4)$, 2nd figure $Ca(NO_3)_2$, 3rd figure $MgSO_4$.

† Dry weights of tops and roots, but not of pods, were computed to a ratio by giving the highest weight in the group the value of 1.00 and reducing others to decimal parts of this value.

nitrate nitrogen in this series. The nitrate nitrogen is at a much lower level than Clements (7) found in his work, resulting entirely from the difference in analytical methods. The only value of the data on nitrate nitrogen is to indicate the reserve supply in the plant, and this is naturally much higher with a short light exposure.

The content of organic nitrogen was entirely inconsistent with the supply of nitrogen in the medium. Here again the light duration determined the level of assimilation. No evidence of a correlation between potassium supply and organic nitrogen synthesis was found.

The pods produced under 17-hour and 13-hour light exposures in series I were also analyzed. The data were of interest only because they showed the high level of carbohydrate content in the partly mature fruit (average of 33.64 per cent. in long light and 29.77 per cent. in intermediate light), but they failed to disclose any consistent relationship with treatment. The organic nitrogen content (average of 3.25 per cent. in long light and 3.22 per cent. in intermediate light) failed to show even the effect of light exposure.

Series II was a repetition of series I, so far as the general plan of the experiment was concerned. Starch and hemicellulose were determined separately, but are reported together as acid-hydrolyzable carbohydrates. The average content of starch was very low, and did not justify separate discussion. The results are given in table IV.

TABLE III

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS

SERIES I

TREATMENT	SIMPLE SUGARS	SUCROSE	TOTAL SUGARS (RATIO)	STARCH	HEMICELLULOSE	NON-SUGARS (RATIO)	TOTAL CARBOHYDRATES		ORGANIC NITROGEN	NITRATE NITROGEN
							PERCENTAGE	RATIO		
	17 hours light									
	%	%		%	%		%		%	%
1–1–6	5.31	3.40	0.99	2.99	3.03	1.00	14.73	0.94	3.17	0.150
1–6–1	3.12	1.77	0.56	1.85	2.62	0.74	9.36	0.59	3.39	0.186
5–1–2	3.31	4.23	0.89	1.74	4.17	0.98	13.45	0.85	3.31	0.105
3–3–2	2.77	5.40	0.93	0.64	4.65	0.87	13.48	0.86	3.42	0.222
2–2–4	4.93	3.80	1.00	1.22	4.80	1.00	15.75	1.00	3.27	0.163
	13 hours light									
1–1–6	3.74	3.49	1.00	2.69	2.70	0.85	12.62	0.99	3.51	0.192
1–6–1	2.69	2.82	0.75	1.77	3.09	0.76	10.37	0.81	3.80	0.745
5–1–2	3.05	3.35	0.88	3.50	2.86	1.00	12.76	1.00	3.06	0.072
3–3–2	3.88	3.27	0.99	2.34	1.94	0.67	11.43	0.89	3.66	0.655
2–2–4	3.41	2.51	0.82	2.55	2.54	0.80	11.01	0.86	3.21	0.368
	10 hours light									
1–1–6	2.50	2.74	0.95	0.84	2.73	0.81	8.81	0.89	4.56	0.268
1–6–1	2.30	2.09	0.79	0.93	2.90	0.87	8.22	0.83	4.67	0.698
5–1–2	2.91	1.87	0.86	0.97	3.28	0.97	9.03	0.91	4.68	0.388
3–3–2	1.22	3.90	0.93	0.73	3.53	0.97	9.38	0.95	4.74	0.400
2–2–4	2 76	2.76	1.00	0.83	3.55	1.00	9.90	1.00	4.82	0.505

TABLE IV

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS
SERIES II

Treatment	Simple sugars	Sucrose	Total sugars (ratio)	Acid-hydrolyzable carbohydrates		Total carbohydrates		Organic nitrogen	Nitrate nitrogen
				Percentage	Ratio	Percentage	Ratio		
	17 hours light								
	%	%		%		%		%	%
1–1–6	10.34	4.00	1.00	7.81	1.00	22.15	1.00	3.071	0.006
1–6–1	10.75	1.91	0.88	6.78	0.87	19.44	0.88	3.487	0.188
5–1–2	5.73	2.19	0.55	7.52	0.96	15.44	0.70	3.241	0.006
3–3–2	6.56	2.30	0.62	6.50	0.83	15.36	0.69	3.527	0.215
2–2–4	9.15	2.21	0.79	6.81	0.87	18.17	0.82	3.402	0.003
	13 hours light								
1–1–6	8.23	5.40	1.00	5.67	0.45	19.30	1.00	3.967	0.004
1–6–1	1.66	4.19	0.43	12.74	1.00	18.59	0.96	3.793	0.119
5–1–2	3.88	2.76	0.49	11.33	0.88	17.97	0.93	3.770	0.025
3–3–2	5.55	2.20	0.57	10.21	0.80	17.96	0.93	3.712	0.005
2–2–4	5.95	4.40	0.76	7.10	0.56	17.45	0.90	3.961	0.005
	10 hours light								
1–1–6	3.29	1.39	0.91	5.83	0.63	10.51	0.80	4.843	0.211
1–6–1	4.49	0.57	0.98	7.86	0.85	12.92	0.97	4.711	0.215
5–1–2	3.45	0.57	0.78	9.20	1.00	13.22	1.00	4.657	0.381
3–3–2	4.20	0.94	1.00	6.85	0.74	11.99	0.90	4.622	0.437
2–2–4	3.59	0.92	0.88	5.15	0.56	9.66	0.73	4.653	0.400

The general level of carbohydrate accumulation in this series was above that of series I. As mentioned previously, perhaps this was a response to the higher air temperatures in the greenhouse. In this series there was not a clear distinction between the levels of carbohydrate accumulation in long and in intermediate light exposures, the temperature factor apparently overshadowing the light effect.

The effect of high magnesium on the amount of sugars found was similar to that in series I, although only treatment 1–1–6 was able to maintain a consistently high level, treatments 2–2–4 and 3–3–2, the latter in particular, being somewhat erratic except in short light. In this series the sugar content of the high potassium culture was lower than in the high calcium treatment. The high level of non-sugars in the potassium cultures is an evident corollary of the low sugar content, as rapid transformation must have occurred.

The lowest content of polysaccharides was found in the highest magnesium treatment, if the ratios of the three light treatments are averaged. It is hardly safe to conclude that this is a fixed relation, however, as the cultures varied widely in this respect.

No one treatment was greatly superior in total carbohydrate accumulation, although the high magnesium treatment led in this respect in long and in intermediate light. The very high percentages found with all treatments tended to mask any effects due to nutrients.

The nitrogen data showed almost no differences between nutrient treatments at any light duration. The light effect, *per se*, was again distinctly shown.

In the third series it was decided to grow the plants only until the first blossoms appeared, to determine whether the differences could be observed before the plants were mature. It was also decided to limit the light durations to 10 and 13 hours, as the greater differences had been found between these durations. Triplicate cultures, involving the growth of 120 plants under each condition, were used, instead of the duplicate cultures in the previous series.

Analytical results of this series are presented in table V. In this table the column "Acid-hydrolyzable carbohydrates" represents only one analytical step, no attempt being made to separate starch from hemicellulose.

The level of carbohydrate accumulation at this stage of the plant's growth was distinctly lower in the 13-hour exposure than was the case when the plants were grown to maturity. With 10-hour exposure, the final level was more nearly like that found in series I.

The highest level of sugar accumulation was found with treatment 2–2–4, as an average of both light durations. Treatment 1–1–6 was unaccountably low in these fractions, while the high potassium treatment was

TABLE V

CONTENT OF CARBOHYDRATE FRACTIONS AND NITROGEN IN TOPS

SERIES III

TREATMENT	SIMPLE SUGARS	SUCROSE	TOTAL SUGARS (RATIO)	ACID-HYDROLYZABLE CARBOHYDRATES		TOTAL CARBOHYDRATES		ORGANIC NITROGEN	NITRATE NITROGEN
				PERCENTAGE	RATIO	PERCENTAGE	RATIO		
	10 hours light								
	%	%		%		%		%	%
1–1–6	2.07	0.45	0.66	6.09	0.91	8.61	0.81	4.511	0.072
1–6–1	2.42	0.25	0.70	5.48	0.82	8.15	0.77	4.459	0.330
5–1–2	2.59	0.37	0.77	5.70	0.85	8.66	0.82	4.414	0.150
3–3–2	3.61	0.23	1.00	6.69	1.00	10.53	1.00	4.345	0.136
2–2–4	2.87	0.16	0.79	6.25	0.93	9.28	0.88	4.259	0.030
	13 hours light								
1–1–6	2.04	0.66	0.77	4.59	0.73	7.29	0.76	5.203	0.358
1–6–1	2.21	0.39	0.74	5.57	0.89	8.17	0.85	5.064	0.427
5–1–2	2.63	0.71	0.98	6.23	1.00	9.57	1.00	4.842	0.188
3–3–2	2.32	0.08	0.69	4.44	0.71	6.84	0.71	4.861	0.299
2–2–4	3.23	0.37	1.00	5.81	0.93	9.41	0.98	4.605	0.261

very good in the short light. The last treatment was again conducive to non-sugar formation in this series, although not superior to treatment 2–2–4. These two treatments were also best in total carbohydrates.

The content of organic nitrogen was the highest in each case in the high magnesium treatment. The differences were only a few hundredths of 1 per cent. and are hardly worthy of mention. The light effect on the level of protein elaboration was noticeable even at this stage of growth.

A general summation of the results of these studies, and a consideration of average ratios, show that the culture solutions might be ranked as follows with respect to production of sugars: 1–1–6, 2–2–4, 3–3–2, 5–1–2, 1–6–1. This indicates that the proportion of magnesium sulphate in the culture solution, indicated by the third figure in each group, bears considerable relation to sugar content. The treatment high in calcium nitrate was rather consistently low in these fractions, while high potassium occupied an intermediate position.

With respect to polysaccharides, the following ranking seems justified: 5–1–2, 1–6–1, 2–2–4, 3–3–2, 1–1–6. Only in the case of the high potassium treatment was there a sharp distinction, as it was greatly superior in nearly all cases.

Total carbohydrates, as mentioned previously, tended to be at about the same level whether the solution was high in potassium or in magnesium. They were clearly lower in the high calcium treatment, excepting in series II in which an excessively high level prevailed under all conditions.

Base element relationships

The material for this study was the oven-dried samples of the tops. No analyses of pods were made. Data on crude ash for all series are given in table VI.

The average amount of crude ash was greater under 10-hour light duration than under the 13-hour in every case. Long light duration was not consistent, being the lowest in series I and the highest in series II.

The effect of nutrient materials on the crude ash was clearly shown. In every case except one, the lowest figure was found with the high magnesium treatment, 1–1–6. The highest percentage of crude ash was noted with the high potassium or high calcium treatment, or a combination of the two, in all groups. This is in accordance with the relative percentages of the three elements commonly found in plants.

Table VII presents the analyses of the three dominant bases in the tops for all series.

The data for series I are also presented in graphic form in figure 1. In this graph the increments of potassium (represented by the first of the three figures) increase from left to right. The increments of calcium (rep-

TABLE VI

PERCENTAGE OF CRUDE ASH IN PEA PLANTS

Treatment*	Percentage crude ash		
	Series I	Series II	Series III
	17 hours light		
	%	%	%
1–1–6	12.71	15.35	.....
1–6–1	12.76	15.91	.....
5–1–2	15.47	18.05	.....
3–3–2	14.59	17.37	.....
2–2–4	14.88	16.85	.....
	13 hours light		
1–1–6	13.41	12.80	11.80
1–6–1	15.48	15.00	16.43
5–1–2	15.21	15.85	17.08
3–3–2	16.43	14.12	14.09
2–2–4	16.55	14.71	13.20
	10 hours light		
1–1–6	14.95	14.96	15.31
1–6–1	16.32	16.71	16.09
5–1–2	16.10	18.22	14.38
3–3–2	15.05	15.77	15.12
2–2–4	15.97	14.97	15.25

* Culture solution, Shive's 1.00-atmosphere: 1st figure KH_2PO_4, 2nd figure $Ca(NO_3)_2$, 3rd figure $MgSO_4$.

resented by the second figure) and of magnesium (represented by the third figure) decrease from left to right. To illustrate more clearly the repressive effect of potassium or the other two bases, the treatment 5–1–2 was placed at the right of all sections of the graph.

Considering first the correlation between the supply of any one base in the culture solution and the percentage in the plants produced in that solution, it can be seen that a very good agreement existed. The only exceptions were the potassium content of treatment 3–3–2 grown in 13-hour light duration and the higher magnesium increments with the same exposure.

Evidence on the mutually repressive effect of the bases was clearly apparent. A high proportion of potassium in the culture solution repressed the calcium intake under all conditions to a greater extent than did a large amount of magnesium. Similarly solution 1–6–1, high in calcium, invariably produced plants with a higher magnesium content than did solution 5–1–2, high in potassium, although the latter supplied twice as much mag-

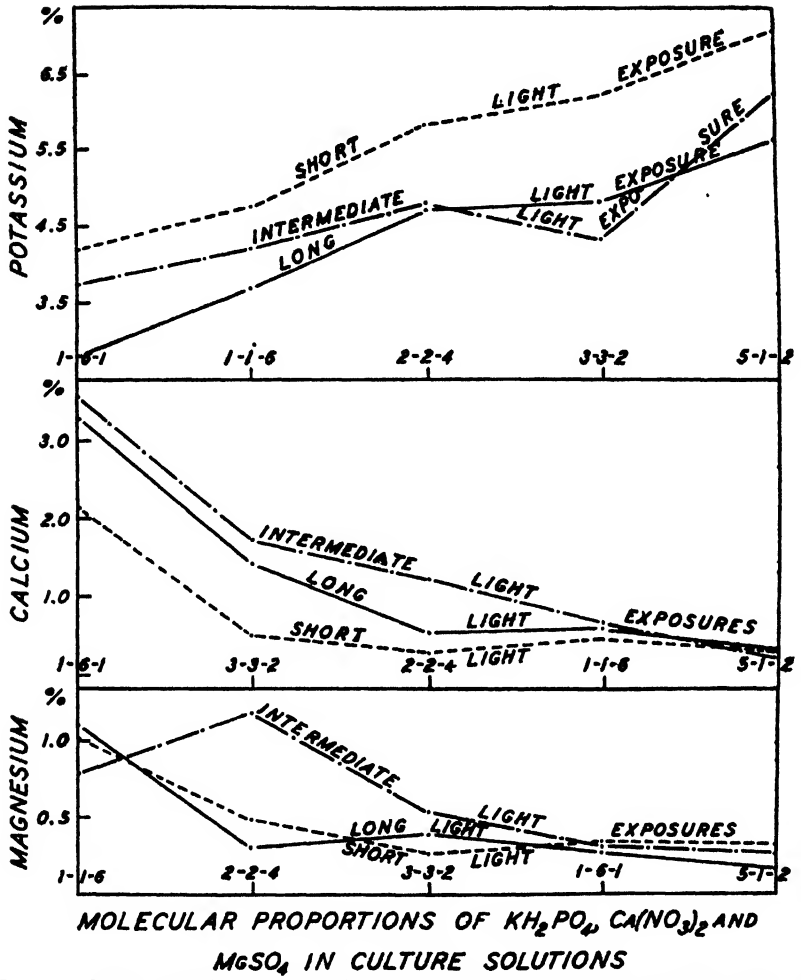


FIG. 1. Graph showing intake of base elements under several light exposures in series I.

nesium. Calcium showed a greater repressive effect than magnesium on the intake of potassium. In all cases solution 1–6–1 produced plants lower in potassium content than did solution 1–1–6.

The most marked effect of the duration of light on the absorption of bases was upon the intake of potassium. With the 10-hour light period the level of potassium intake was significantly higher throughout the range of cultural treatment. An intermediate photoperiod gave higher percentages of this element than long light, with the exception of one treatment. Between the extreme treatments an average difference of over 1 per cent. in potassium content was found.

TABLE VII

CONTENT OF POTASSIUM, CALCIUM, AND MAGNESIUM IN TOPS OF PEAS

TREATMENT	PERCENTAGE ON OVEN-DRY BASIS								
	SERIES I			SERIES II			SERIES III		
	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg
	17 hours light								
	%	%	%	%	%	%	%	%	%
1–1–6	3.64	0.58	1.13	4.26	0.36	1.50	.....	.....	.....
1–6–1	2.75	3.32	0.27	4.01	2.81	0.40	.....	.....	.....
5–1–2	5.64	0.27	0.15	6.50	0.24	0.27	.....	.....	.....
3–3–2	4.81	1.45	0.41	5.47	0.77	0.90	.....	.....	.....
2–2–4	4.69	0.54	0.30	5.16	1.40	1.37	.....	.....	.....
	13 hours light								
1–1–6	4.20	0.61	0.80	5.05	0.29	0.92	5.01	0.35	0.99
1–6–1	3.67	3.61	0.33	4.75	2.16	0.31	5.14	1.26	0.35
5–1–2	6.38	0.19	0.27	7.05	0.16	0.19	6.38	0.20	0.30
3–3–2	4.37	1.70	0.53	6.37	1.09	0.26	5.86	0.28	0.36
2–2–4	4.83	1.25	1.19	5.48	0.63	0.43	4.95	0.33	0.64
	10 hours light								
1–1–6	4.76	0.47	1.00	5.90	0.26	0.60	6.54	0.28	0.56
1–6–1	4.20	2.18	0.33	5.76	1.36	0.30	6.29	1.01	0.32
5–1–2	7.10	0.22	0.32	8.10	0.16	0.23	6.81	0.20	0.27
3–3–2	6.27	0.51	0.28	6.96	0.37	0.35	5.41	0.24	0.29
2–2–4	5.87	0.29	0.50	6.51	0.28	0.52	5.77	0.28	0.46

Calcium content in reference to duration of light showed the 13-hour day length able to maintain the highest level. Short light duration was featured by the lowest relative level of calcium absorption, and except in the highest concentration by a rather low absolute content.

With respect to magnesium the intake was not consistent. Intermediate light duration permitted a greater intake than long light in all but one treatment. Under 10-hour day length the range of magnesium content was narrower, the low magnesium cultures having a slightly higher intake and the high concentrations being somewhat less than under greater light durations.

The data for series II also are presented in table VII, and shown graphically in figure 2. The correlation between the supply present and the absorption was considerably better in most respects. The calcium content of the plants grown in solution 3–3–2 under maximum light exposure was lower than in solution 2–2–4, but this was the only inconsistency in this respect in the series.

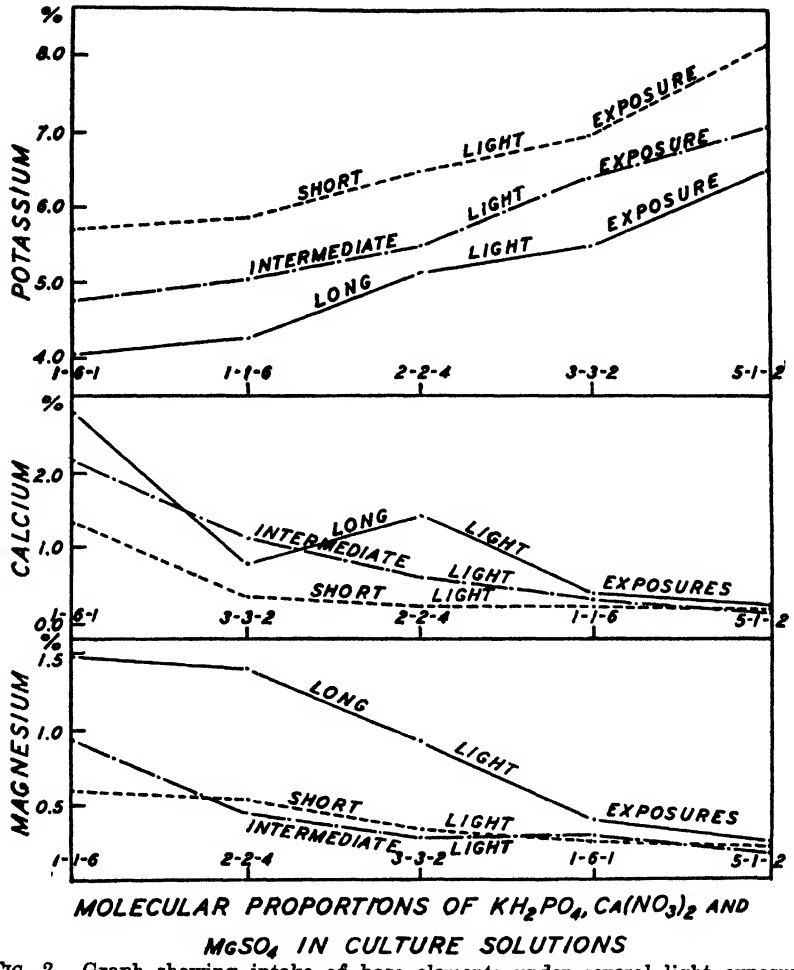


FIG. 2. Graph showing intake of base elements under several light exposures in series II.

The various repressive effects noted in series I were again evident in this series. The high potassium culture, 5–1–2, was particularly repressive to calcium and magnesium absorption in all cases.

Very definite differences in potassium absorption with respect to light duration were found. Plants subjected to a 10-hour exposure were again the highest in potassium content, while intermediate exposure was clearly more conducive to potassium intake than long exposure. As might be expected in view of the antagonism of potassium and calcium, the short exposure plants had the lowest level of calcium intake. The differences between long and intermediate light periods were not so conclusive, but favored the former in all but one treatment.

Magnesium was considerably higher under long light exposure than in series I, and definitely greater than in the shorter light exposures of this series.

Because the plants in the third series were grown for a period of only four weeks, the results as shown in table VII and figure 3 are not entirely comparable with those of the preceding series. The response to high or to low supply of the different elements was not so great, consequently the range of percentages was much reduced. A fairly high level of potassium content was reached but calcium and magnesium were very low. The exceedingly low magnesium content of the young leaves was in agreement with results of LUTMAN and WALBRIDGE (18) on potatoes analyzed at several stages of growth. Potassium was somewhat antagonistic to the other bases, but the relatively greater repressive effect of calcium toward potassium had not begun to be evident.

The effect of light duration was more clearly shown in the content of calcium and magnesium than in that of potassium. Both bases were absorbed in direct relation to the light period. Apparently the absorption of these elements was rather regular during the early stages of growth, and no great accumulation took place in any culture. On the other hand, the potassium was not taken up in accordance with the supply, at least in the

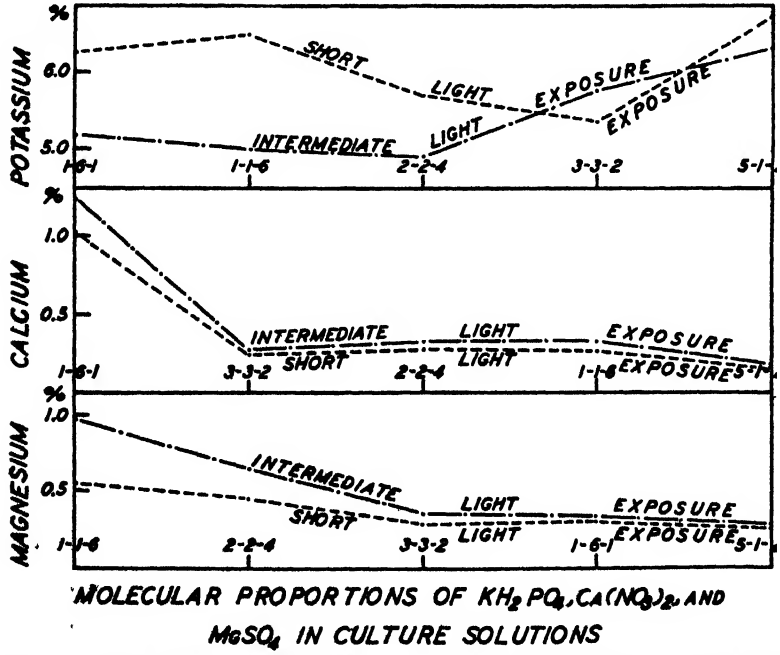


FIG. 3. Graph showing intake of base elements under several light exposures in series III.

lower increments. Only in the solution containing the largest amount of potassium was there an abundant intake. In the other cultures, one part of potash in the solution seemed to permit as great an intake as three. This would indicate that a lower concentration of potash is adequate for young plants.

Considering the evidence of the first two series, it seems conclusive that potassium was most abundant in plants grown to maturity under a 10-hour light exposure. As the light period was lengthened the level of potassium intake dropped. In young plants these phenomena were not so well defined.

On the other hand, calcium was most abundant under longer light periods, with 13-hour and 17-hour durations about equally efficient in promoting absorption of this element. Magnesium was similarly affected by the photoperiod.

In attempting to explain the results of these experiments, it is well to remember that on these same plants, determinations of organic and total nitrogen showed that the shorter the light period, the higher the percentage of nitrogen. This was in accordance with the findings of many other investigators. Moreover the length of day was practically the only controlling factor, as the plants were able to get an adequate supply of nitrogen from the lowest increments, and therefore failed to respond to increased amounts.

Thus it may be assumed that, in a series of culture solutions having the same osmotic concentration but varying proportions of the three salts, all the plants in the series would have an equal supply of base elements. It is generally agreed that an acid-base equilibrium exists in plants. If light conditions were such as to permit a heavy intake of nitrates, an equivalent amount of one or more bases must also enter the plant. In these experiments, potassium was apparently available in the greatest relative amount; hence its high level in the short-light plants which were all equally high in organic nitrogen. It is also significant that it was with short light only that nitrate nitrogen was present to any amount in these plants. In experiments on the same general theme, HIBBARD (**14**) found an accumulation of nitrogen in the plants grown in short light, proportional to the amount of calcium. In this case calcium was apparently the most abundant cation, although no analyses of plants are presented to show whether the plants had absorbed more calcium.

The absorption of calcium, and to a large extent of magnesium, seems to bear a reciprocal relation to that of potassium. The statements of HOAGLAND and MARTIN (**15**) in reference to toxicity of salts under various climatic conditions are of interest in connection with these studies. They found that solutions which stimulated growth under certain conditions might produce marked inhibition of growth at a different time of year.

Summary and conclusions

1. Nott's Excelsior field peas were grown in selected water cultures, SHIVE's 1.00 atmosphere, type 1. Analyses of tops for carbohydrate fractions, organic and nitrate nitrogen, crude ash, potassium, calcium, and magnesium are reported. The effect of light duration on the relative amounts of these plant constituents was studied. Physical measurements are given in all cases.

2. Results of physical measurements indicate that the highest average top length, dry weight of tops, and dry weight of entire plants exclusive of fruit were found with solutions high in calcium nitrate. With respect to the last measurements, a high potassium treatment was more effective than were high magnesium treatments.

3. Highest content of total sugars (simple sugars and sucrose) was found with solutions high in magnesium. Polysaccharides (starch and hemicellulose) was markedly high with an abundant supply of potassium.

4. Total carbohydrates were maintained at a fairly high level by all treatments except the one high in calcium nitrate. This is in direct contrast to the findings of CLEMENTS (7) for long light conditions, but in partial agreement with his analyses for shorter light periods, and in good agreement with earlier findings.

5. No consistent difference in content of organic nitrogen due to difference in culture solution was found in any case. This finding also contrasts with the results reported by CLEMENTS, who found that a high potassium supply was correlated with high total nitrogen in the plant.

6. Crude ash was higher in plants grown in a 10-hour than in a 13-hour light period, but not consistently higher than in plants grown in a 17-hour duration. A culture solution high in magnesium resulted in the lowest crude ash in all but one case. High potassium or high calcium solutions grew plants which were the highest in crude ash.

7. Light exposures of 10 hours daily produced plants high in potassium and low in calcium and magnesium.

8. Light exposures of 13 hours produced plants lower in potash and higher in calcium and magnesium than 10-hour plants. This exposure in series I had the highest level of calcium intake.

9. Light exposures of 17 hours resulted in plants markedly low in potash and usually the highest in calcium and magnesium.

10. The correlation between the relative supply of any one element in a group of cultures and the relative intake of that element by the plants grown in these cultures was clearly marked.

11. The mutually repressive effect of the three bases was demonstrated. Potassium was so strongly repressive as to overshadow other factors when

the supply of this element was high and the supply of the other bases rather low.

The writer is indebted to Dr. R. P. HIBBARD for guidance during the course of the experiments, to Dr. H. F. CLEMENTS for suggestions in planning the work, and to Dr. P. J. ANDERSON for permission to complete the necessary analytical work.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
WINDSOR, CONNECTICUT

LITERATURE CITED

1. ADIE, R. H., and WOOD, T. B. A new method of estimating potassium. Jour. Chem. Soc. **77**: 1076–1080. 1900.
2. ANDERSON, P. J., SWANBACK, T. R., and STREET, O. E. Potash requirements of the tobacco crop. Connecticut Agr. Exp. Sta. Bull. 334. 1932.
3. ARTHUR, J. M., GUTHRIE, J. D., and NEWELL, J. M. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930.
4. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 2d ed. Washington, D. C. 1925.
5. BARTHOLOMEW, R. P., and JANSSEN, G. The rate of absorption of potassium by plants and its possible effect upon the amount of potassium remaining in soils from applications of potassium fertilizers. Arkansas Agr. Exp. Sta. Bull. 265. 1931.
6. BRENCHLEY, W. E. Inorganic plant poisons and stimulants. Cambridge University Press, Cambridge. 1914.
7. CLEMENTS, H. F. Plant nutrition studies in relation to the triangular system of water cultures. Plant Physiol. **3**: 441–458. 1928.
8. FONDER, J. F. Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. Jour. Amer. Soc. Agron. **21**: 732–750. 1929.
9. GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.
10. ————, and ————. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871–920. 1923.
11. ————, BACON, C. W., and ————. Photoperiodism in relation to hydrogen-ion concentration of the cell sap and the car-

bohydrate content of the plant. Jour. Agr. Res. **27**: 119–156. 1924.

12. HAAS, A. R. C. Effect of nitrate salts upon growth and composition of tobacco leaves. Bot. Gaz. **88**: 96–102. 1929.
13. HANDY, J. O. The volumetric determination of magnesium. Jour. Amer. Chem. Soc. **22**: 31–39. 1900.
14. HIBBARD, R. P. The influence of calcium and potassium deficiencies on the transformation and utilization of carbohydrates and proteins in the pea. Michigan Agr. Exp. Sta. Bienn. Rept. 17–18. 1930.
15. HOAGLAND, D. R., and MARTIN, J. C. Effect of salts on the intake of inorganic elements and on the buffer system of the plant. Univ. California Agr. Exp. Sta. Tech. Paper 8. 1923.
16. HOLTZ, H. F., and LARSON, C. A simple method for nitrate nitrogen determination in wheat plants. Plant Physiol. **4**: 288–291. 1929.
17. LIVINGSTON, B. E., KELLERMAN, K. F., and CROCKER, W. A plan for cooperative research on the salt requirements of representative agricultural plants. Rept. Com. Div. Biol. Agr. Nat. Res. Council. Baltimore. 1919.
18. LUTMAN, B. F., and WALBRIDGE, N. L. The rôle of magnesium in the aging of plants. Vermont Agr. Exp. Sta. Bull. 296. 1929.
19. MCHARGUE, J. S. The rôle of manganese in plants. Jour. Amer. Chem. Soc. **44**: 1592–1598. 1922.
20. MAZÉ, P. Détermination des éléments minéraux rares necessaires au développement du maïs. Compt. Rend. Acad. Sci. (Paris) **160**: 211–214. 1915.
21. MORGAN, M. F. The soils of Connecticut. Connecticut Agr. Exp. Sta. Bull. 320. 1930.
22. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
23. SAYRE, C. B., WILLAMAN, J. J., and KERTESZ, Z. I. Factors affecting the quality of commercial canning peas. New York (Geneva) Agr. Exp. Sta. Tech. Bull. 176. 1931.
24. SHIVE, J. W. A three-salt nutrient solution for plants. Amer. Jour. Bot. **2**: 157–160. 1915.
25. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant culture. Physiol. Res. **1**: 133–145. 1914.
26. TYSON, J. Influence of soil conditions, fertilizer treatments, and light intensity on growth, chemical composition, and enzymic activities of sugar beets. Michigan Agr. Exp. Sta. Tech. Bull. 108. 1930.

APPLICATION OF CALORIMETRIC METHODS TO ECOLOGICAL RESEARCH

FRANCES L. LONG

(WITH TWO FIGURES)

The method of determining the heat of combustion by means of an oxygen bomb calorimeter has been adapted to plant studies of energy relations. This is based on standard chemical and physical practise, so that results obtained by one worker can be compared with those obtained by another. In general, it is the method by means of which heating values for coal and other fuels are obtained; the procedure that is used for determinations of caloric values for fats, proteins, and sugars upon which diets are based.

Prompt drying of plant material at moderate temperatures immediately after collecting is essential to accuracy, owing to the destructive effects of respiration and enzyme action. This demands an oven with considerable capacity, special ventilation, and uniformity of temperature throughout. An oven to meet these requirements was built for this work and is described in the paper by MARTIN.[1] The thermo-regulator is set for 75° C., since this temperature, aided by air currents, affords the most favorable condition for drying plant material of the type used here, with the minimum risk of destructive effects during the process. After drying, the material is ground fine, thoroughly mixed, and then made into pellets by means of a pellet press. To prevent these pellets from absorbing atmospheric moisture, which makes them so large and loose that they may fall from the capsule, they must be kept in a desiccator until required for use.

In calorimeters of the usual type having outside walls of bakelite or other insulating material, there is a heat interchange between the interior of the instrument and the surrounding air. Although the best insulation possible is used, it is still necessary to take heat losses into consideration and to make corrections for radiation to secure accurate results. The calorimeter used for the present studies was so constructed as to secure adiabatic conditions within the apparatus, thus avoiding heat leakage and the consequent necessity for corrections. The interior of the instrument, or calorimeter proper, is completely surrounded by jacketing walls through which water is circulated from a cold water tap and hot water boiler, so that the temperature can be regulated during the determination. By maintaining the jacket at a temperature corresponding with that of the calorimeter, all heat losses due to radiation are eliminated.

[1] MARTIN, E. V. Improved drying oven for plant material. Plant Physiol. 9: 1934.

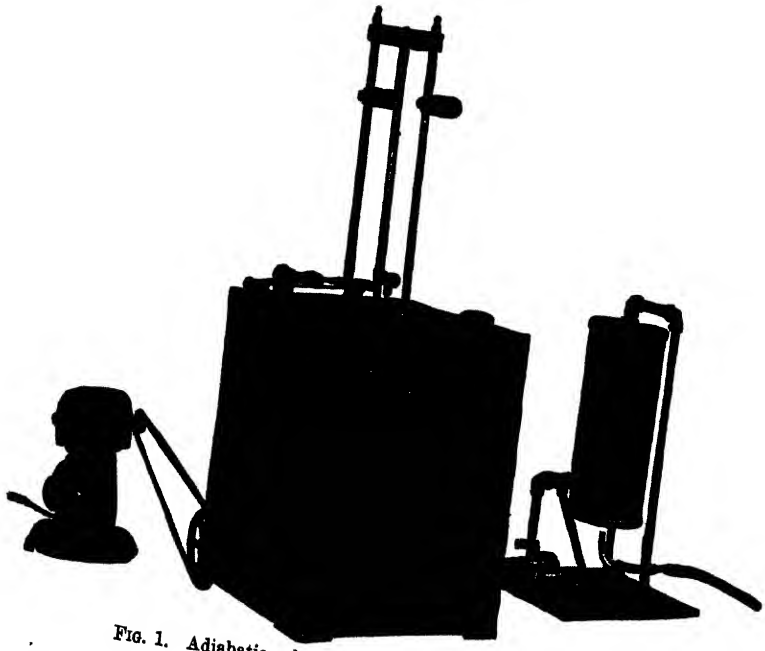


FIG. 1. Adiabatic calorimeter with motor and water heater.

Within the jacket is a metal casing, oval in form, which is so fitted as to allow a minimum air space between it and the oval bucket which is placed within it. The bucket is nickel-plated and highly polished to decrease thermal exchanges by radiation. It holds the 2 liters of water, the stirrer, and the thermometer.

For the determination, the pellets are weighed in a weighing bottle and then placed in a capsule which is suspended in the bomb. Both the bomb and the capsule are made of illium alloy, which resists the action of the nitric and sulphuric acids formed as a result of combustion. One of the necessary conditions is the avoidance of secondary reactions involving the oxidation or solution of the material of which the bomb is made.[2]

The fuse wire is adjusted so that it makes contact with the terminals and pellets. Then the illium bomb is filled with oxygen to 25 lb. pressure, placed in the calorimeter bucket and covered with 2 liters of distilled water. The lid to the calorimeter is pushed forward and closed, and the motor started in order to stir the water surrounding the bomb and jacket. When the switch is closed that makes the electrical connection, the fuse ignites

[2] For specific directions and details see Booklet 106, The oxygen bomb calorimeter. Standard Calorimeter Co., East Moline, Illinois.

the pellets in the capsule and is itself burned. From the rise of temperature of the water in the bucket and the grams dry weight of plant material in the pellets used, the number of calories of energy involved is calculated.

It is necessary to make corrections for the heat produced by the fuse wire, which for the kind of wire employed is 2.4 calories per cm. Because of the use of pure oxygen at high temperature and pressure, certain reactions take place that do not occur in the ordinary process of combustion. Thus the free nitrogen in the small amount of residual air present upon closing the instrument is partially oxidized to N_2O_5, which combines with the moisture present in the bomb to become HNO_3. Similarly the nitrogen of the plant material oxidizes to a greater or less extent to HNO_3. The sulphur in the pellets, which under ordinary conditions of combustion is oxidized to SO_2, is converted in the calorimeter to SO_3, and this to H_2SO_4.

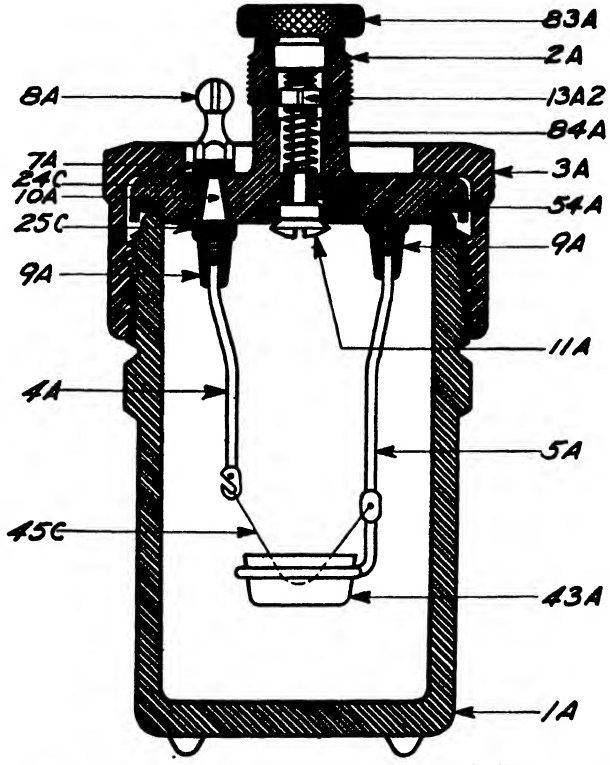


FIG. 2. Details of illium bomb: 1 A, illium cylinder; 2 A, illium cover, held in place by 3 A; 3 A, steel screw cap; 4 A, 5 A, electric terminals for fuse wire; 7 A, top terminal insulator; 8 A, top terminal; 9 A, illium locknut; 10 A, terminal cone; 11 A, illium valve; 13 A2, valve nut; 43 A, illium capsule for pellets; 54 A, cover gasket; 83 A, valve thumb nut; 84 A, valve spring; 24 C, cone insulator; 25 C, mica washer; 45 C, special fuse wire.

Since these acids represent a small portion of the energy produced, the bomb is rinsed out with distilled water, and the washings titrated with a standard solution of sodium carbonate made up of such a strength that each cc. represents one calorie. The calculations for a typical determination are shown in the example given here.

— Example —

$$\frac{\text{Water equivalent of calorimeter} \times \text{temp. rise °C.} - \text{corrections (wire + acid)}}{\text{Grams dry weight pellets}} = \text{calories per gram dry weight}$$

$$\frac{2450 \times 1.61 - 22.9}{1.1625} = 3365 \text{ calories per gram dry weight.}$$

The water equivalent of the calorimeter represents the amount of heat absorbed by the whole apparatus, including the 2 liters of water, per degree rise of temperature. It is found by combusting a standard substance of known heat value. The substances most commonly used with the values recognized by the United States Bureau of Standards are:

Benzoic acid	6320	calories per gm.
Naphthalene	9622	" " "
Cane sugar	3949	" " "

The water equivalent for the calorimeter used for the preceding example was found to be 2450. Unless otherwise stated, the small calorie is used here. It represents the amount of heat required to raise one cc. of water through 1° C. A large calorie is the equivalent of 1000 small calories.

The values in large calories for some of the organic compounds contained in plants were found to be as follows: Para rubber 10.2, resin 8.4, pure cellulose 4.0, and starch 3.8. Since there are many forms of resin, rubber, etc., these values must be considered as specific only for the plants from which they were taken, but they do represent the general range of values to be found in such materials. In consequence, when determinations show high caloric values it is usually an indication that the material contains an unusual amount of hydrocarbons, since each gram of these compounds has more than twice the value of cellulose, sugar, starch, and protein, the other principal components of plant tissues.

The number of grams that may be combusted at one time is determined not only by the size of the illium capsule, but also by the caloric value of the material. Three pellets weighing a total of 1 to 2 gm. make a convenient amount for each combustion. The thermometers used with this calorimeter read from 65° to 90° F., in 1/20 degree divisions. Two grams of *Helianthus annuus* seed when combusted in the calorimeter, give a rise of about

10° F., which would go beyond the limits of the thermometer if the initial temperature were above 80° F.; hence a safe rule is to limit the amount of material for each combustion to a maximum of 2 gm. and to have the initial temperature of the water within the thermometer range.

After combustion, the oxides of the mineral salts in the plant are found in the capsule as ash. In the giant cactus (*Carnegiea gigantea*), the ash of certain parts of the plant may be as high as 25.5 per cent. of the dry weight. Leaves of *Mesembryanthemum aequilaterale* contain about 25 per cent. of ash, while those of *Rosa, Rubus*, and *Prunus* yield approximately 6 per cent. ash on the same basis. In cases where the caloric value is not of special interest, but where it is desirable to know the amount of ash in various plant parts, the determinations may be made as indicated without recording the temperature rise. Ash analyses may also be made from the residue in the capsules when that is desirable. For example, table I gives WOLFF'S[3] analyses for the ash of *Helianthus annuus*.

TABLE I

COMPOSITION OF ASH OF *HELIANTHUS ANNUUS* IN PERCENTAGE

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_2	SiO_2	Cl
	%	%	%	%	%	%	%	%	%
Seeds and fruits	16.23	7.41	7.63	12.29	1.6	35.43	2.34	14.65	2.42
Whole plant	60.77	1.84	12.56	6.75	0.22	8.89	1.71	0.88	6.38

Calorimetric determinations were made upon seeds of a number of species and life forms in order to ascertain the energy stored in each and to permit a comparison of the relative caloric values. The task of drying seeds before preparing the pellets is much simpler than with more succulent material, since respiration is at a minimum and the material is comparatively dry at the start. Combustion proceeds readily enough when whole seeds are used, but better average results were obtained from pellets of ground material. Table II gives the results obtained for the seeds of a number of crop plants and native conifers.

The great differences in growth during the early seedling stages, when the plant is dependent on the food stored in the seed, are more readily understood when one compares the energy supply upon which these plants may draw. For instance, *Pinus lambertiana* averaged 1222 calories per seed, 50 times as much as in *Pinus contorta murrayana*. The average for *Ricinus communis* was 2975 calories, or 2.4 times that of sugar pine and 20 times as much as for seeds of *Avena sativa*.

[3] WOLFF, EMIL. Aschen Analysen. 1871.

TABLE II

CALORIC AND ASH VALUES FOR VARIOUS SEEDS

SPECIES	CALORIES PER GM. DRY WEIGHT	CALORIES PER SEED	ASH
			%
Avena sativa	4238.3	143.4	2.5
Zea mays	4415.2	.....	.....
Sorghum vulgare	4017.6	144.6	1.2
Triticum sativum	4282.3	.....	0.8
Phaseolus coccineus	4282.2	.....	.....
Medicago sativa	5068.9	.....	.....
Cannabis sativa	5890.7	122.9	3.1
Brassica nigra	6049.5	.....	3.6
Helianthus annuus	6759.2	.....	.....
Ricinus communis	6834.7	2975.6	2.3
Pseudotsuga taxifolia	5998.3	59.9	1.5
Pinus ponderosa	5625.0	192.4	2.7
Pinus contorta murrayana	5989.2	26.7	2.7
Pinus lambertiana	6480.4	1221.8	1.7
Pinus flexilis	7117.7	372.9	2.6

The calorimetric method has been applied on an extensive scale to the plants obtained from the transplant and adaptation experiments carried on at the Alpine Laboratory and at Santa Barbara. Some representative results drawn from these are given in tables IV–IX. In addition, various series of determinations have been made for purposes of orientation, a typical example of which is furnished in table III, which records the caloric values for the largest individual of a widely spread group of cultivated sunflowers. At the time this plant was used, the achenes of the single large head were mature but not dry, while the lower leaves were slightly torn and beginning to turn brown. The leaves were cut in the order of position and labeled; they were weighed at once and the area taken by means of blue prints before drying. The stem, which was 2 meters tall, was cut into small pieces after weighing to insure rapid drying in the oven, and root and flower head were treated likewise. Each of these yielded much more material than needed for pellets, but an aliquot of each was utilized to secure a representative result (table III).

There proved to be less uniformity in the area, weight, and caloric value of adjacent leaves than was expected from their general appearance, a diversity probably due partly to shading and partly to competition between the leaves. Weights and calories rose more or less irregularly from a minimum at the bottom to a maximum in the upper third, to fall off rapidly to the top. By contrast, water gave the highest values in the lower third,

TABLE III

COMPARATIVE VALUES OF LEAVES AND ORGANS OF *HELIANTHUS ANNUUS*

LEAF	LEAF WEIGHT		WATER	ASH	LEAF AREA ONE SIDE	CALORIES PER GM. DRY WEIGHT	CALORIES IN WHOLE LEAF	RELATIVE VALUES
	WET	DRY						
	gm.	*gm.*	%	%	*sq. cm.*			
1 (top)	15.5	3.7	76.1	12.7	337	3993.2	14.780	43.8
2	19.6	4.3	78.0	12.4	339	3999.4	17.200	51.0
3	18.6	3.8	79.6	12.5	426	3971.0	15,100	44.8
4	38.8	8.2	78.9	11.4	770	3934.1	32,250	95.6
5	30.1	5.2	82.7	10.5	651	4051.6	21,100	62.5
6	36.1	7.5	79.3	11.8	709	4017.5	30,100	89.2
7	40.1	8.1	79.8	11.1	820	3907.9	31,600	93.6
8	24.7	4.5	81.8	13.7	526	3932.7	17,700	52.5
9	42.8	8.6	80.0	12.0	798	3922.7	33,750	100.0
10	29.9	5.5	81.6	12.5	629	4040.6	22,230	65.9
11	25.9	5.2	80.0	12.1	509	3935.2	20,740	60.6
12	33.7	6.6	80.5	12.7	648	3936.3	25,970	76.9
13	20.9	3.8	81.7	11.7	494	3896.3	14,800	43.8
14	29.3	5.7	80.6	12.8	583	3881.2	22,130	65.5
15	27.2	5.4	80.2	13.4	536	3821.4	20,650	61.2
16	17.4	2.9	83.3	13.4	420	3912.1	11,350	33.6
17	17.5	3.2	81.8	14.7	416	3866.9	12,390	36.7
18	17.1	3.0	82.5	14.8	403	3826.7	11,490	34.0
19	17.1	2.3	86.5	14.9	393	3742.4	8,610	25.6
20	16.2	2.6	84.0	15.7	392	3662.3	9,530	28.2
21	19.3	4.4	77.2	14.3	440	3769.6	16,600	49.2
22	12.8	2.4	81.2	16.6	296	3720.2	8,350	24.9
23	14.3	2.9	79.6	16.6	350	3557.7	13,310	39.4
24	11.9	2.3	80.6	16.4	283	3520.7	8,100	24.0
25	11.3	2.1	81.5	17.4	274	3546.7	7,450	22.1
26	8.2	1.4	82.9	17.9	220	3424.8	4,795	14.2
27	9.1	1.7	81.3	18.7	234	3497.6	5,950	17.6
Average	22.4	4.35	80.9	13.9	480	3825.5	16,954	
Whole plant	3638.8	578.8	82.8	8.8		3983.5	2,320,964	100.0
Leaves	605.4	117.3	80.9	13.9	12,956	3825.5	457,725	19.7
Stem	1342.0	204.4	84.5	7 3	195 cm. high	3850.0	786,940	33.9
Root	419.4	87.5	79.2	8 8		3951.0	345,713	14.8
Head	1272.0	169.6	86.6	5.2	30 cm. diam.	4307.7	730,586	31.6

TABLE IV

RELATION OF TOTAL CALORIES TO INTENSITY OF COMPETITION

SPECIES	DRY WEIGHT PER PLANT	WATER	ASH	CALORIES PER GM. DRY WT.	TOTAL CALORIES PER PLANT	RELATIVE VALUES
	gm.	%	%			
Helianthus annuus **(young)**						
4 normal	11.8	89.2	15.3	3807	44,923	100.0
16 "	9.5	86.0	15.0	3701	35,160	78.2
64 "	9.8	83.6	15.4	3664	35,907	79.9
128 "	7.2	90.5	16.7	3600	25,920	57.7
Helianthus annuus **(mature)**						
4 normal	352.5	83.2	8.9	3835	1,351,838	100.0
16 "	119.0	83.2	7.5	3934	468,146	34.6
16 suppressed	54.6	83.5	7.6	3897	212,776	15.7
64 normal	59.8	84.1	10.2	3813	228,017	16.8
64 suppressed	1.2	91.1	9.4	3819	4,583	0.3
128 normal	45.7	84.5	8.5	3799	173,614	12.8
128 suppressed	16.1	79.0	9.6	3717	59,844	4.4
Triticum sativum **(young)**						
4 normal	4.8	83.1	14.3	3585	17,208	100.0
16 "	3.4	83.3	25.1	3491	11,869	68.9
64 "	2.3	81.6	25.5	3463	7,965	46.2
Triticum sativum **(mature)**						
4 normal	65.5	66.0	4.2	4042	264,751	100.0
16 "	50.5	63.0	3.5	4103	207,202	78.2
64 "	11.6	58.0	8.6	3828	44,405	16.7
128 "	8.4	54.0	14.5	3594	30,190	11.4
Zea mays **(young)**						
4 normal	42.3	80.0	10.8	4006	169,454	100.0
16 "	20.7	86.3	12.3	3868	80,068	47.2
64 "	24.3	84.3	10.9	3955	86,107	50.8
128 "	18.1	86.4	8.2	3747	67,821	40.0
Zea mays **(mature)**						
4 normal	241.7	84.9	3.5	4053	979,610	100.0
16 "	99.5	75.2	5.1	3961	394,120	40.0
16 suppressed	27.2	72.7	4.1	3899	106,053	10.8
64 normal	44.3	78.1	4.6	3909	173,169	17.6
64 suppressed	10.6	80.7	10.1	3828	40,577	4.1
Campanula medium						
4 normal	310.7	76.0	10.4	4498	1,397,529	100.0
64 "	37.0	78.0	10.6	3834	141,969	10.1
Plantago lanceolata						
4 normal	283.7	74.0	15.0	3628	1,029,264	100.0
64 suppressed	5.2	70.0	19.4	3507	18,236	1.7

and ash at the bottom, the latter decreasing rather regularly and the former rather irregularly to the top. The caloric value per gram was highest in the head, owing to the storage of oil in the seeds.

For the study of adaptation under different degrees of competition, a number of cultures have been established each year, with frequent changes of the species employed. Each culture contained four units 4 ft. square, separated by paths 1.5 ft. wide, in which the respective densities were 4, 16, 64, and 128 plants. The bed with four individuals served as a standard for the other three, in each of which the density was sufficient to produce a smaller or larger number of suppressed plants (table IV).

As would be expected; the young plants showed less response to crowding, since the demands of each individual had not reached the maximum. The mature plants yielded consistent and striking results throughout, the three crop species giving nearly identical relative values in the two higher densities. The suppressed plants were naturally more variable, but for the most part averaged about a fourth of the value for the normal or dominant individuals.

In all competition cultures without a buffer margin, the outermost plants profit from the lack of competition on the edge, the individuals at each corner often being especially well grown. Conditions as to water content and nutrients are much the same on all four sides, but the light intensity is distinctly higher on the south (table V).

The plants on the south margin were approximately 2.5 times higher in calories than were those on the north, primarily as a consequence of

TABLE V

TOTAL CALORIES PRODUCED UNDER COMPETITION AT SAME DENSITY

HELIANTHUS ANNUUS	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
	WET	DRY					
	gm.	*gm.*	%	%			
South margin	938.2	152.8	83.7	7.1	4071	622,049	100.0
North margin	416.1	61.5	84.9	8.1	3925	242,003	38.9
North suppressed	261.4	34.0	87.0	7.8	4018	136,612	21.9
Center dominant	121.1	21.3	82.4	7.8	3855	82,112	13.0
Center suppressed	83.1	9.8	85.8	8.0	3751	36,560	5.8

better illumination, while dominant plants on the north were about twice as large as the suppressed ones. The caloric values in the center were distinctly less for all individuals, while the suppressed were less than half as large as the dominants and contained but 5 per cent. as many calories as the plants on the south margin.

In seeking the functional explanation of the striking modifications and transformations secured under low light intensities, the calorimeter has proved an effective adjunct to measurements of transpiration and photosynthate. The species listed in table VI were grown in lath houses with light values of 12 to 4 per cent. of sunlight, the controls being located in sunshine.

The various species differ strikingly in their efficiency under the reduced intensities. The highest values in light of 12 per cent. were for *Solanum, Verbesina,* and *Mentzelia,* with relative performances of 15, 14, and 13 approximately, while the lowest were for *Allium, Madia,* and *Helianthus,* all at about 1 per cent. The best relative production in 4 per cent. light was that of *Clarkia* at 7.4, followed closely by *Solanum* at 6.4; the poorest performance again was that of *Allium, Madia,* and *Helianthus,* with half or less of 1 per cent. The average production in the deeper shade was 2.7, in the lighter shade 6.3, these values being in fair agreement with the respective light intensities. The number of calories may also vary greatly from year to year in correspondence with climatic conditions, especially sunshine and temperature, as is evidenced by the results obtained with *Helianthus* and *Plantago* in successive years.

In the endeavor to ascertain the effects of different lengths of day on species growing in the experimental garden, dark tents were employed to reduce the time of exposure to daylight from 13 hours in the long-day series to 9 in the mid-day and 5 in the short-day series. The plants were regularly best-grown in the longer exposure, and flowering occurred about two weeks earlier, leaves and flowers both being deeper colored. The mid-day series was intermediate in all respects, except that the stems were tallest. In the short-day tents the plants were shortest and palest, the flowers fewer, smaller and paler, blooming later than in either of the other series. In all cases the results were similar to those obtained in the lath houses, and this fact is reflected in the values shown in table VII, although the exposure to direct sunlight gave higher amounts in practically all cases.

The relative values differ much for the different species, the highest being given by *Verbesina* and the lowest by *Zinnia,* thus reflecting their abilities to grow and become modified in the shade. In general terms, the average production in the mid-day series with two-thirds as much sunshine was a little more than one-third of that in the long-day; and in the short-day with four hours less, it was again slightly more than one-third.

TABLE VI

RELATION OF TOTAL CALORIES TO LIGHT INTENSITY

SPECIES	LIGHT VALUES	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
	%	*gm.*	*gm.*	%	%			
Allium cepa	Sun	348.0	35.0	90.0	11.1	3777	132,195	100.0
	12	3.1	0.22	93.0	16.9	3762	817	1.0
	4	1.7	0.13	92.0	20.6	3552	462	0.6
Clarkia elegans	Sun	225.0	45.0	80.0	5.8	3980	179,100	100.0
	12	16.5	3.8	74.0	9.7	3821	14,520	8.7
	4	19.0	3.6	79.0	8.1	3757	13,525	7.4
Gilia capitata	Sun	317.5	81.5	74.0	6.0	4204	342,626	100.0
	12	12.1	2.3	81.0	7.4	4074	9,370	2.7
	4	4.2	0.9	79.0	14.1	3835	3,452	1.0
Helianthus annuus (1930)	Sun	950.6	217.7	77.1	5.7	3996	869,929	100.0
	12	25.0	2.1	91.6	10.4	3645	7,655	0.8
	4	5.5	0.16	97.1	10.4	3625	580	0.06
Helianthus annuus (1931)	Sun	602.0	149.2	75.0	4.5	3955	590,086	100.0
	12	52.3	6.1	88.0	12.8	3593	22,527	3.8
	4	8.7	1.2	86.0	16.6	3348	4,018	0.6
Madia elegans	Sun	1,260.0	394.1	69.0	8.5	4175	1,645,368	100.0
	12	24.9	5.3	79.0	18.6	3464	18,359	1.1
	4	11.0	2.3	79.0	20.5	3286	7,558	0.4
Mentzelia lindleyi	Sun	179.1	24.0	86.8	14.6	3581	88,344	100.0
	12	35.3	3.5	90.1	21.0	3323	11,631	13.1
	4	7.6	1.0	86.9	26.4	3110	3,110	3.5
Plantago lanceolata (1930)	Sun	149.3	22.4	85.0	14.3	3641	81,558	100.0
	12	11.1	1.4	87.4	13.7	3502	4,903	6.1
	4	10.7	0.9	92.0	19.0	3368	3,031	3.5
Plantago lanceolata (1931)	Sun	520.0	133.0	74.0	11.3	3826	508,858	100.0
	12	46.1	4.1	91.0	21.5	3202	13,128	2.5
	4	34.9	3.1	91.0	21.5	3127	9,694	1.8
Rumex patientia	Sun	279.3	58.8	79.0	11.3	3834	225,439	100.0
	12	16.1	3.9	76.0	18.3	3707	14,457	6.4
	4	7.2	1.0	86.0	19.4	3681	3,681	1.6
Solanum tuberosum	Sun	200.7	50.1	75.0	13.3	3819	191,332	100.0
	12	84.3	8.8	90.0	20.1	3417	30,070	15.6
	4	42.2	4.1	91.0	24.1	3169	12,993	6.7
Verbesina encelioides	Sun	540.0	125.7	77.0	10.3	3788	476,152	100.0
	12	95.7	19.3	80.0	15.3	3433	66,257	13.9
	4	48.8	6.7	86.0	21.2	3082	20,649	4.0

TABLE VII

RELATION OF TOTAL CALORIES TO LENGTH OF DAY

SPECIES	LIGHT	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
		gm.	*gm.*	%	%			
Gilia capitata	Long-day	317.5	81.5	74.0	6.0	4204	342,626	100.0
	Mid-day	181.5	36.7	80.0	11.1	3705	135,974	39.7
	Short-day	55.5	11.0	80.0	19.0	3482	38,302	11.1
Phacelia grandiflora	Long-day	223.0	58.0	74.0	18.5	3446	199,868	100.0
	Mid-day	60.5	13.3	78.0	20.4	3311	44,042	22.0
	Short-day	38.0	8.5	78.0	26.4	3094	26,297	13.1
Rumex patientia	Long-day	279.3	58.8	79.0	11.3	3834	225,439	100.0
	Mid-day	102.0	27.5	73.0	12.4	3774	103,785	46.0
	Short-day	21.8	3.8	83.0	15.6	3688	14,014	6.2
Verbesina encelioides	Long-day	117.4	25.3	78.5	11.5	3701	93,653	100.0
	Mid-day	142.1	20.7	85.4	11.5	3563	74,100	79.1
	Short-day	71.7	9.3	87.1	13.8	3488	32,439	34.6
Zinnia grandiflora	Long-day	88.0	18.8	79.0	11.3	3809	71,600	100.0
	Mid-day	9.8	2.4	75.0	13.9	3567	8,561	11.9
	Short-day	8.8	1.6	82.0	21.0	3247	3,247	4.5

TABLE VIII

RELATION OF TOTAL CALORIES TO AMOUNT OF NUTRIENTS

SPECIES	NUTRIENT RATIO	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT	RELATIVE VALUES
		WET	DRY					
		gm.	*gm.*	%	%			
Calendula officinalis	2N: 2W	694.5	120.0	83.0	11.9	3871	464,520	100.0
	1N: 1W	166.5	37.5	78.0	16.2	3742	140,325	30.2
	0N: 0W	100.5	18.2	82.0	17.0	3608	65,666	14.1
Gilia capitata	2N: 2W	635.1	185.5	71.0	5.8	4268	791,714	100.0
	1N: 1W	102.5	28.7	72.0	5.1	4282	122,893	15.5
	0N: 0W	68.1	18.2	73.0	5.7	4205	76,531	9.5
Madia elegans	2N: 2W	253.5	103.0	59.0	14.7	3950	406,850	100.0
	1N: 1W	110.0	45.5	59.0	13.6	3801	172,946	42.5
	0N: 0W	68.5	21.6	68.0	18.9	3519	76,010	18.6
Petunia hybrida	2N: 2W	56.7	10.5	82.0	14.4	3747	39,344	100.0
	1N: 1W	55.5	9.8	82.0	18.8	3548	34,770	90.7
	0N: 0W	8.8	1.7	80.0	24.8	3564	6,059	15.8
Solanum tuberosum	2N: 2W	235.0	58.5	75.0	9.5	3932	230,022	100.0
	1N: 1W	121.0	26.9	78.0	12.5	3805	102,355	44.4
	0N: 0W	42.0	8.8	79.0	16.2	3783	33,290	14.4

In the series designed to test the response to increased nutrients, commercial fertilizer was applied to one row of beds in the amount regularly used by gardeners for the soil concerned, and in twice the amount. The supply of water was similarly increased to maintain the soil solution at about normal density, while in the control row no addition was made to the amount received from rainfall, and no nutrient was added (table VIII).

As was to be expected, the addition of nutrients and water greatly increased the size of the plants in comparison with the controls, the number of shoots being greater and the leaves larger and more deeply colored. The difference between the several species was marked, the maximum effect being found in *Gilia* with 11 times the control and the minimum in *Madia* with 5.5 times.

The soil series consisted of soil pits in three rows, one containing fertile garden loam, another a fertile black clay called adobe, and a third, sand. While soil is the remote factor, the immediate response is to water content, the fluctuations of which are automatically regulated by soil texture under the condition of equal watering (table IX).

TABLE IX

RELATION OF TOTAL CALORIES TO TYPE OF SOIL

SPECIES	SOIL	WEIGHT OF PLANT		WATER	ASH	CALORIES PER GM. DRY WT.	CALORIES PER PLANT
		WET	DRY				
		gm.	*gm.*	%	%		
Madia elegans	Loam	1,260.0	394.1	69.0	8.5	4175.0	1,645,368
	Adobe	422.0	182.5	57.0	8.1	4105.0	749,163
	Sand	275.5	111.8	60.0	14.9	3973.0	433,000
Plantago lanceolata	Loam	480.0	106.4	78.0	8.3	4008.2	426,472
	Adobe	334.5	70.7	79.0	13.5	3709.4	263,254
	Sand	65.0	9.6	85.0	13.6	3319.8	31,870
Solanum tuberosum	Loam	200.0	50.1	75.0	13.3	3819.4	191,352
	Adobe	30.0	7.5	75.0	6.8	3809.5	28,580
	Sand	15.8	1.3	80.0	7.4	3795.6	4,930

In all three species selected, the growth in loam was two to several times that in the adobe, and growth in the latter two to eight times that in the sand. In general the soil series yielded the most consistent and striking modifications in response to varying water content.

From the preceding data it is evident that the calorimetric method introduces into ecology and the related sciences, agriculture, horticulture, forestry, etc., an accurate chemical procedure for measuring performance. It makes it possible to ascertain in exact terms not only how much energy is utilized, but also how much is stored in seed, bud, or underground part

against the time of future need. The efficiency of the individual plant, variety, or species may be compared with that of others grown under the same conditions, and thus serve as a definite basis for selection or breeding. The plant itself may be employed as an index of the energy available at different altitudes or latitude, under various natural canopies or artificial covers, or in connection with long-day and short-day exposures, as in the work of GARNER and ALLARD. It also becomes possible to obtain the plant's reaction to the influence of varying amounts of water, in the case of irrigated crops for example, of soil nutrients and fertilizers, of the air content in the case of water-logged soils, and so forth. The effect of the time and intensity of grazing range grasses or time of cutting fields of alfalfa can be measured with signal accuracy, and perhaps even a forecast made of the next season's growth on the basis of the amount of stored food.

Furthermore, two processes of fundamental importance in plant life, competition in the community and adaptation in the individual, can be evaluated more accurately and objectively by means of the calorimeter. The degree of competition between plants determines not only the amount of energy available for each, but also the conditions under which it can be utilized, while the energy actually transformed represents the performance of the several competitors.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

CARBON DIOXIDE CONTENT OF THE GAS FROM PEA PODS

Z. I. KERTESZ

(WITH ONE FIGURE)

Introduction

Shelled green peas deteriorate very rapidly. Respiration (**10**) and enzymes (**7, 8**) are mostly responsible for these objectionable changes, which cause a material loss in many canneries. Several studies on the prevention of this deterioration have been reported (**8, 9, 14**); but in spite of the well known fact that peas stored in the pods (*i.e.*, in a natural gas mixture) keep better than shelled peas, no method has been proposed for the storage of shelled green peas in a gas.

Gas storage of fruits and vegetables has been studied by several investigators (**9, 15**). Experiments with the storage of shelled green peas in a gas similar to that contained by the pods may offer a possibility for the prevention of deterioration of the peas between shelling and canning. The writer presents the results of investigations on the composition of the gas contained in the pods of green cannery peas (*Pisum sativum* L. variety Perfection).

Earlier workers (**11**, p. 391) were of the opinion that the systematic arrangement of plant organs precludes a deficiency of oxygen in plants, and held that carbon dioxide is absent in them (**2**, III, p. 19). Other investigators proved this assumption faulty, however, and found carbon dioxide in the different organs of plants. LUMIA (**2**, III, p. 19) showed that gas samples obtained from unripe figs contained 5.25 per cent. carbon dioxide and only 17.92 per cent. oxygen. NEGRI (**2**, III, p. 19) found 9.88 per cent. carbon dioxide and 16.59 per cent. oxygen in the gas from immature fruits of *Gomphocarpus*, while the gas from the ripe fruits contained 3.48 per cent. carbon dioxide and 23.15 per cent. oxygen. DEVAUX (2, III, p. 20) analyzed the air from the hollow inside of a pumpkin (*Cucurbita maxima*) and found 2.52 per cent. carbon dioxide, 18.29 per cent. oxygen, and 79.19 per cent. nitrogen. MALAQUIN (**2**, III, p. 19) found in the gas from the pods of *Colutea*, 6.9 per cent. carbon dioxide and 14.3 per cent. oxygen. MAGNESS (**13**) observed that the gas in the intercellular spaces of apples contains the following percentages of carbon dioxide: About 7 per cent. when stored at 6° C., 12 per cent. at 11° C., 17 per cent. at 20° C., and 21 per cent. at 30° C. At the same time there was a corresponding decrease in the oxygen content of the samples. He found the same general relation to exist in potatoes and carrots. DAVIS (**5**) found in the gas samples from potatoes a carbon dioxide content of 5–6 per cent. and 10–11 per

cent. of oxygen when they were stored at 17°–18° C. With increasing storage temperature the carbon dioxide content and the respiratory ratio enlarged rapidly. Recently DANIN (4) studied the gas contained in the cenobia of *Rivularia polyotis*, and found that light intensity influenced the oxygen content, which reached 70 per cent. under certain circumstances. COLLA (3) investigated the carbon dioxide and oxygen contents of different organs of *Nymphaea alba*, and found an increased oxygen content in the roots and stems as a result of illumination. During the night a decrease in oxygen content of the gas in the leaves was observed, while only insignificant changes in the carbon dioxide content occurred. In the roots, however, he found a decrease in the carbon dioxide content during the day and an increase at night.

Besides the normal constituents of air, such as oxygen, nitrogen, and carbon dioxide, the presence of other gases in plant organs has been reported also. CLOËZ and GRATIOLET, BOUSSINGAULT, and CORENWINDER (2, I, p. 522) were unable to find carbon monoxide in the plants studied by them, while LANGDON and GAILEY (12) showed the presence of carbon monoxide in the gas obtained from the pneumatocyst of the Pacific coast kelp, *Nereocystis leutkeana*. The presence of hydrocarbons (methane ?) in plants has also been reported (2, I, p. 522).

It is evident, therefore, that carbon dioxide is often present in the gas contained in plants or in plant cavities. No information could be obtained from the literature on the composition of the gas contained in pea pods.

Experimentation

PRELIMINARY WORK AND METHODS USED

The carbon dioxide content of the gas contained in the pods of samples of commercial peas shipped from California to Geneva, New York, was first determined. The samples were kept under constant refrigeration during shipment and were removed from the containers immediately upon arrival. The temperature of the peas was between 5° and 10° C.

The method used in the determinations was as follows: A separatory funnel of 100 cc. capacity was filled with water and placed with the tip upward in a large beaker filled with water. A common glass funnel was placed in the opening of the separatory funnel and under this the peas were shelled. The gas from the opened pea pods bubbled up through the funnel into the separatory funnel. After all the pods of a sample were opened and the gas collected, the funnel into the opening of the separatory funnel was removed and replaced by a beaker of about 200 cc. capacity. This beaker was placed under the separatory funnel while the latter was still submerged in the water in the large beaker. The separatory funnel and the small beaker were then lifted out of the large beaker and carried to the

gas analysis apparatus. The whole operation was usually completed in three minutes.

The gas sample was sucked over into a Hempel precision gas burette, using potassium hydroxide to absorb the carbon dioxide and alkaline pyrogallol solution to absorb the oxygen present in the gas samples. All determinations were made at 26°–29° C., and no correction was made for temperature, which would affect only the values of "gas per pod." The results of the preliminary determinations are presented in table I.

TABLE I

CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS SHIPPED FROM CALIFORNIA TO GENEVA, N. Y. (CALIFORNIA TELEPHONE PEAS)

SAMPLE NO.	DATE	CO_2	O_2	O_2 CALCULATED	DIFFERENCE IN O_2	RESPIRATORY RATIO
		%	%	*cc.*	*cc.*	
1	April 23	0.91	20.40	21.00	0.60	1.52
2	April 28	0.52	19.96	21.00	1.04	0.48
3	May 13	0.72	19.78	21.00	1.22	0.56

As expected, the gas obtained from the pea pods contained carbon dioxide, the percentage varying from 0.52 to 0.91. Since the method for collecting the gas samples gave satisfactory duplicate results in several trials, it was used for all determinations reported here.

In table I, as also in several following tables, values are presented for the respiratory quotient. The calculation of these values is based on the assumption that pea pods contain normal air, and that the rate of diffusion for the different gases through the pods is the same. As far as the writer is aware, no data on the diffusion of different gases through pea pods have been published.

In the calculation of the quotients it was assumed that the air contained in the pods was of normal composition, containing 21 per cent. of oxygen by volume and 79 per cent. of other constituents (mostly nitrogen), and that the latter was not absorbed by the analytical solutions. From the residual amount of this fraction from the gas samples was calculated the amount of oxygen associated with it originally. This amount of oxygen is referred to as "O_2 calculated." The difference between this calculated amount and the amount of oxygen actually determined is referred to in the tables as "difference in oxygen." It might be said that this difference in oxygen represents the efficiency in oxygen determined in the pods at a certain time and not the amount of oxygen consumed during a certain period.

The ratio between the amount of carbon dioxide found and the difference in oxygen is designated in the tables as "respiratory ratio," although it must be realized that it represents the findings *at* a certain time and not the ratio of oxygen consumed and carbon dioxide produced *during* a certain period.

This ratio showed a wide variation in the samples used for preliminary determinations, but on account of the unknown origin and history of these samples no conclusions are drawn from the figures.

Changes in composition of gas of pea pods associated with growth

To obtain an insight into the changes that might be related to the growth and ripening of peas in the pods, several samples were collected at 8 A.M. at different dates during the 1932 season, from the same field of Perfection peas in Geneva, N. Y. These peas were sown on April 10 and maximum blooming occurred on June 20. On each harvest date large numbers of pods were picked and representative samples of 35 to 71 pods were chosen for analysis. The results of the determinations are presented in table II.

The average weight of pods increased during the early growth stages and then slowly decreased. This is in accordance with the findings of Bisson and Jones (**6**) on Dwarf Telephone peas. The amount of gas obtained per pod remained nearly constant in spite of the considerable changes in the size of the pods and of the peas in the pods. The percentage of carbon dioxide in the gas samples obtained was also fairly constant.

The changes in the sugar content of growing peas have been studied by several investigators (**1, 6, 8**). All have found that the maximum sugar content is attained at a relatively early stage of ripening, much before the peas are considered ripe for canning. The decrease in sugar content continues until the peas are dry. The rate of respiration decreases in growing peas presumably as a result of the diminishing sugar content. On the basis of these facts, therefore, it was reasonable to suppose that the carbon dioxide content in the pea pods would decrease during ripening, but the results presented in table II show no such essential change. This may be explained by assuming a changing rate of diffusion of the gases through the pods, depending upon the relative concentration of carbon dioxide inside. While the rate of respiration undoubtedly changes during growth, the rate of absolute or specific diffusion seems to be regulated by some factors in such a manner that a roughly constant carbon dioxide content is maintained in the pods. The respiratory quotient varied from 0.34 to 0.57.

Diurnal changes in carbon dioxide content of pea pods

The fact that no change in the carbon dioxide content of the gas in pea pods during growth could be established raised the question as to whether

TABLE II

CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS AS INFLUENCED BY CHANGES IN GROWTH; ALL SAMPLES HARVESTED AT 8 A. M.

SAMPLE NO.	DATE (JULY)	SIZE OF PODS	AVERAGE LENGTH	NO. OF PODS	AVERAGE WEIGHT OF PODS	TOTAL GAS OBTAINED	TOTAL GAS PER POD	CO_2	O_2	CALCULATED O_2	DIFFERENCE IN O_2	RESPIRATORY RATIO
			mm.		*gm.*	*cc.*	*cc.*	%	%	*cc.*	*cc.*	
1	3	Medium	59	45	3.54	45.30	1.01	1.55	18.56	9.63	1.23	0.57
2	5	Medium	..	71	....	67.00	0.95	1.72	18.14	14.28	2.13	0.54
3	5	Large	68*	53	5.76	46.70	0.88	1.61	16.93	10.11	2.21	0.34
4	6	Large	....	53	5.49	61.20	1.15	1.88	17.90	13.05	2.10	0.55
5	13	Large	.....	35	4.47	39.55	1.13	1.14	. . .	...	. . ...	.....
		Average	.....	....	.....	..... .	1.02	1.58	17.88	.. ..	.. ..	0.50

* Cannery ripe.

the carbon dioxide content varied over a 24-hour period. One set of determinations, presented in table III and in figure 1, proved that marked changes in the carbon dioxide content do occur during a 24-hour day.

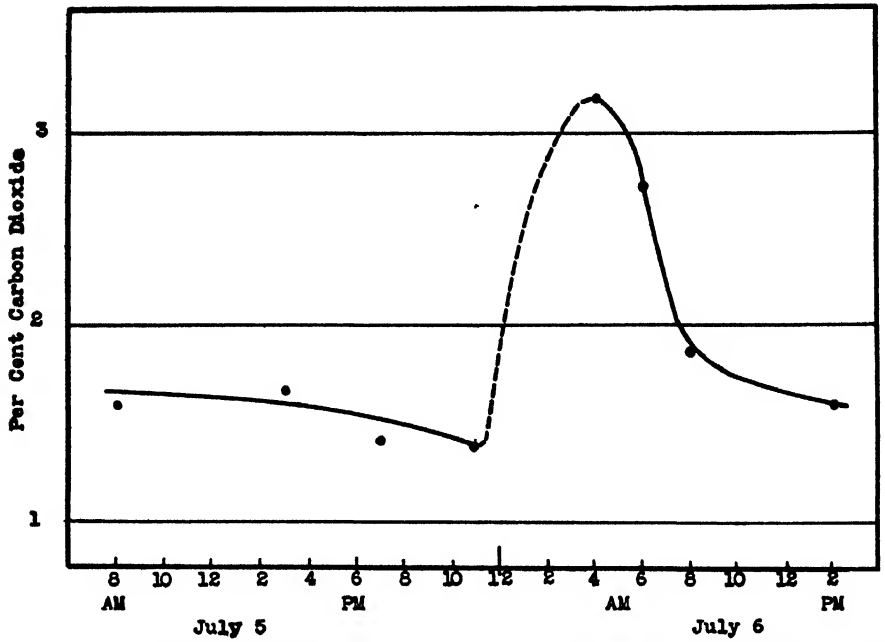


FIG. 1.—Changes in carbon dioxide content of gas from pea pods during the day and at night.

For these experiments Perfection pea pods were used from the same field from which all the material for the other experiments (except the preliminary ones) was collected. The average weight of a single pod varied from 5.19 to 5.95 grams, giving a mean weight of 5.62 grams. The "gas per pod" was about the same as in the previous experiment, ranging around 1 cc.

The percentage of carbon dioxide in the gas sample taken from pods at 8:00 A. M. on July 5 was 1.61. This value decreased somewhat later in the day, reading at 11:00 P. M. 1.39 per cent. Unfortunately no determinations were made between 11:00 P. M. and 4:00 A. M. next day, when the percentage of carbon dioxide was 3.19. On July 6 the values for the carbon dioxide content of the gas decreased rapidly, and by 8:00 A. M. had reached almost the average in table II. During the day it decreased again, as it did on the previous day. The same relation between the time of day and the carbon dioxide content was found by COLLA (3) in the roots of *Nymphaea alba*. It is interesting to note that the

TABLE III

CHANGES IN CARBON DIOXIDE AND OXYGEN CONTENTS OF PEA PODS DURING THE DAY AND AT NIGHT

SAMPLE NO.	DATE (1932)	WHEN TAKEN	NO. OF PODS	AVERAGE WT. OF PODS	GAS OBTAINED	GAS PER POD	CO_2	CO_2	O_2	O_2	CALC. O_2	DIFF. O_2	RESPIRATORY RATIO
				gm.	*cc.*	*cc.*	*cc.*	%	*cc.*	%	*cc.*	*cc.*	
1	July 5	8 A. M.	53	5.76	46.70	0.88	0.75	1.61	7.90	16.93	10.11	2.21	0.34
2		3 P. M.	50	5.84	48.00	0.96	0.80	1.67	8.80	18.35	10.21	1.41	0.57
3		7 P. M.	60	.....	60.20	1.00	0.85	1.41	.....	.....	.....	.....	.....
4		11 P. M.	45	5.26	36.00	0.80	0.50	1.39	6.20	17.22	7.79	1.59	0.31
5	July 6	4 A. M.	53	5.71	50.20	0.95	1.60	3.19	.....	.....	.....	.....	.....
6		6 A. M.	53	5.95	49.40	0.93	1.35	2.73	6.45	13.06	11.06	4.61	0.29
7		8 A. M.	53	5.64	61.20	1.15	1.15	1.88	10.95	17.90	13.05	2.10	0.55
8		2 P. M.	53	5.19	61.70	1.16	1.00	1.62	11.25	18.25	13.15	1.90	0.53
		Average	.....	5.62	.....	0.98	.....	.....	.....	.....	.....	.....	.....

respiratory quotient did not vary in spite of the broad fluctuation of the carbon dioxide content in the gas samples.

The marked changes in the carbon dioxide content of the pods are undoubtedly due to several factors. It is reasonable to suppose that during the night, when translocation of the sugars takes place, the sugar content of the peas increases. This higher sugar content causes an increase in the rate of respiration (11), which results in higher percentage of carbon dioxide in the pods. The lack of photosynthesis during the night may be partly responsible for the higher carbon dioxide content found in the pods. Of course, the absolute or specific permeability of the pods may also change because of the changing temperature and moisture content of the surrounding air. This last factor, however, seems of minor importance in view of the immaterial changes observed during growth, although samples were often collected under widely different meteorological conditions.

Influence of short storage and freezing on carbon dioxide content of pea pods

For this experiment a great number of pods were picked on July 13, 1932, and divided into several groups containing 35 pods each. One group was opened as previously described and the gas analyzed at once, while duplicate samples were stored at + 25°, + 5°, – 5°, and – 20° C. for 18 hours. After this, one sample from each temperature was analyzed, while the other sample was stored at + 25° C. for an additional 6-hour period and then analyzed. This experiment was carried out twice with practically the same results. The results of the second experiment are presented in table IV.

The "gas per pod" always decreased during storage. The carbon dioxide content of the gas samples from the pods stored at room temperature increased up to 5 and 18 hours, but was decreasing again at 24 hours. In the samples stored at lower temperatures for 18 hours the carbon dioxide content was higher than in the samples kept at room temperature.

The frozen samples stored for an additional 6-hour period at room temperature showed further substantial increases in carbon dioxide content. This gain was more pronounced in the sample stored at – 20° than in that kept at – 5° for the 18-hour period. That the carbon dioxide in these samples increased after defrosting must be the result of the freezing, which partially destroys the structure of the peas and increases their rate of respiration.

It was of interest to note what part the permeability of the pods to gases plays in the composition of the gas held inside. For this purpose several samples were sealed with low-melting-point paraffin and stored similarly to those samples reported in table IV. As one would expect, during freezing the paraffin broke away from the frozen pods or was cracked to such an

TABLE IV

INFLUENCE OF STORAGE AND FREEZING ON CARBON DIOXIDE CONTENT OF PEA PODS

SAMPLE NO.	STORAGE CONDITIONS	AVERAGE WEIGHT OF PODS	GAS OBTAINED	GAS PER POD	CO_2
		gm.	*cc.*	*cc.*	%
1	No storage	4.47	39.55	1.13	1.14
2	5 hr. at +25° C.	4.71	30.35	0.87	1.81
3	18 hr. at +25° C.	4.97	27.20	0.78	2.20
4	24 hr. at +25° C.	4.86	26.00	0.74	1.89
5	18 hr. at +5° C.	4.60	31.40	0.90	2.71
6	Same, concluded by 6 hr. at +25° C.	4.82	29.65	0.85	2.19
7	18 hr. at −5° C.	4.59	24.70	0.71	4.46
8	Same, concluded by 6 hr. at +25° C.	4.67	22.45	0.64	10.90
9	18 hr. at −20° C.	4.55	32.05	0.92	4.53
10	Same, concluded by 6 hr. at +25° C.	4.49	30.65	0.88	17.93

extent that perfect closure could not be expected. This was especially true in samples 7 and 8 of table V. To overcome this difficulty one sample (no. 10) was frozen for 18 hours, and sealed only after defrosting at room temperature.

In all samples in which the pods were kept at room temperature for any length of time, the carbon dioxide content was 42.6 to 49.8 per cent. This concentration seems to be the limit for carbon dioxide production, although small amounts of oxygen were still present in the pods. The changed respiratory quotient indicates a disturbed respiration. Higher ratios were observed by several workers in the case of injured peas or unsuitable conditions (**5, 10, 11**).

Summary

1. During the growth of Perfection peas, no significant changes in the carbon dioxide content of the gas in the pods could be found when all of the samples were collected at 8 A.M. The average value was 1.6 per cent.

2. During the night the carbon dioxide content of the pea pods was doubled in the samples studied. The carbon dioxide content of the pods began to decrease as early as 6 A.M., and about 8 A.M. reached a level that was approximately maintained during the rest of the day. It is suggested that this higher carbon dioxide content of the pods during the night is due to the changed ratio of respiration to photosynthesis.

TABLE V

INFLUENCE OF PARAFFIN SEALING ON CARBON DIOXIDE CONTENT OF STORED AND FROZEN PEA PODS

SAMPLE NO.	STORAGE CONDITIONS	GAS OBTAINED	GAS PER POD	CO_2	CO_2	O_2	O_2	CALC. O_2	DIFF. IN O_2	RESPIRATORY RATIO
		cc.	*cc.*	*cc.*	%	*cc.*	%	*cc.*	*cc.*	
1	5 hr. at 25° C.	24.60	0.70	10.60	43.10	0.70	2.85	3.54	2.84	3.73
2	18 hr. at 25° C.	18.80	0.54	8.30	44.20					
3	24 hr. at 25° C.	22.20	0.63	10.00	45.40					
4	18 hr. at +5° C.	23.30	0.67	3.90	16.73					
5	Same, concluded by 6 hr. at +25° C.	22.30	0.64	9.50	42.60	0.95	4.26	3.15	2.20	4.32
6	18 hr. at −5° C.	29.90	0.85	1.90	6.35					
7	Same, concluded by 6 hr. at 25° C. (sealed before freezing)	22.45	0.64	2.45	10.91*					
8	18 hr. at −20° C., concluded by 6 hr. at 25° C. (sealed before freezing)	26.40	0.75	2.85	10.79*					
9	Same, sealed after cold storage, before 6 hr.-period	40.00	1.14	19.90	49.75	0.70	1.75	5.16	4.46	4.46

* Samples imperfectly sealed because during freezing some of the paraffin came off.

3. When pods were frozen, the carbon dioxide content of the gas contained in them increased to 4.5 per cent., presumably on account of the higher respiratory activity caused by freezing. Changed permeability of the pods may also take part in this phenomenon. When the frozen peas were then stored at 25° C., this carbon dioxide increased to a value of 17.9 per cent.

4. When pea pods were sealed with paraffin and stored at room temperature or frozen and kept at room temperature afterward, the carbon dioxide content of the gas from the pods reached 43 to 50 per cent. This indicates that there must be a constant and very vigorous exchange of gases through the normal pods, in order to keep the carbon dioxide content as low as an average of 1.6 per cent.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK

LITERATURE CITED

1. BOSWELL, V. R. Factors influencing yield and quality of peas. Maryland Agr. Exp. Sta. Bull. 306. 1929.
2. CZAPEK, F. Biochemie der Pflanzen. 3rd ed. Gustav Fischer, Jena. 1922–1925.
3. COLLA, SILVIA. Sulle variazioni del contenuto in O_2 e in CO_2 negli organi di *Nymphaea alba* L. Boll. Soc. ital. Biol. sper. **6**: 823–826. 1931. Ber. ges. Physiol. **66**: 575. 1932.
4. DANIN, Z. Gas content of the cenobia of *Rivularia polyotis* (A. Ag.) Hauck. (Trans. title.) Atti R. Accad. Lincei. **15**: 317–322. 1932. Br. Chem. Abstr. 1932. 888.
5. DAVIS, W. B. Physiological investigation of black heart of potato tuber. Bot. Gaz. **81**: 323–338. 1926.
6. BISSON, C. S., and JONES, H. A. Changes accompanying fruit development in the garden pea. Plant Physiol. **7**: 91–105. 1932.
7. KERTESZ, Z. I. The chemical changes in peas after picking. Plant Physiol. **5**: 399–412. 1930.
8. ————, and GREEN, E. L. Deterioration in shelled green peas held a few days in storage prior to canning. Jour. Agr. Res. **45**: 361–370. 1932.
9. KIDD, F., WEST, C., and KIDD, M. N. Gas storage of fruit. Department of Scientific and Industrial Research (British), Special report no. 31. London. 1927.
10. KOHMAN, E. F. New facts relating to quality in canning peas. The Canner (Convention no.) **74**: 64–66. 1932.

11. KOSTYCHEV, S. Chemical plant physiology. Translated by CHARLES J. LYON. Blakiston, Philadelphia. 1931.
12. LANGDON, S. C., and GAILEY, W. R. Carbon monoxide a respiration product of *Nereocystis luetkeana*. Bot. Gaz. **70**: 230–239. 1920.
13. MAGNESS, J. R. Composition of gases in intercellular spaces of apples and potatoes. Bot. Gaz. **70**: 308–316. 1920.
14. SMITH, R. H. Special investigations. Effect of holding vine peas in cold storage. The Canner (Convention no.) **70**: 211–212. 1930.
15. THORNTON, N. C. Carbon dioxide storage of fruits, vegetables and flowers. Ind. and Eng. Chem. **22**: 1186–1189. 1930.

METABOLISM OF ETIOLATED SEEDLINGS AS AFFECTED BY AMMONIUM NUTRITION

LELAND BURKHART

Introduction

Changes in the nitrogen relations of plants caused by altering the nitrogen supply have been studied by numerous investigators. These changes, however, must be correlated with the metabolism of other substances, especially carbohydrates, in order to understand better the nitrogen relationships.

The nitrogenous metabolism of the seedling concerns itself mainly with the decomposition of already existing proteins stored in the kernel, followed by the translocation and regeneration of the simpler substances into proteins at the growing points. A study of etiolated seedlings may be expected to yield information as to the intermediate products of protein metabolism, for protein regeneration is inhibited in the absence of light, owing primarily to insufficient carbohydrate supply.

PRIANISCHNIKOW and coworkers (**7, 8, 9**) have emphasized the importance of available carbohydrates in the nitrogen metabolism of etiolated seedlings supplied with ammonium salts; however, no carbohydrate determinations were made. The seedlings, grown in water cultures for a period of ten days in the dark, were classified into three groups:

I. Seedlings of the grass type (barley, maize) and the pumpkin when supplied with ammonium salts showed increases in total nitrogen and amides, but no increase in ammonia.

II. Seedlings of the starchy legumes (pea, vetch) increased in total nitrogen and amides only when ammonium salts were accompanied by calcium carbonate.

III. Seedlings of yellow lupine showed that nutrition with ammonium salts caused serious disturbances in the synthetic reactions, manifested by accumulation of ammonia and decrease of asparagine. Addition of calcium carbonate failed to restore the normal course of nitrogen metabolism.

The seeds used differ greatly with respect to the relative amounts of nitrogen free and nitrogen containing reserves (**7**). The respective ratios used as an index to these differences are as follows: grass type 6:1, starchy legumes 2:1, and yellow lupine 0.6:1. Artificial types were employed (**7, 14**) from which it was shown that utilization of ammonium salts was not a specific character of the plant, but depended upon the nutritive condition.

One of the chief criticisms concerning PRIANISCHNIKOW'S investigations is the method which he employed in preparing the plant material for analy-

sis. The seedlings were dried at 70° C., thus rendering his ammonia determinations misleading. Furthermore, he did not separate the principal storage organs from the remainder of the seedlings, thereby having no basis for determining the extent of protein decomposition in the seed and regeneration in the growing seedling. HUNTER (3), employing improved methods of chemical analysis, including carbohydrate determinations, carried out experiments similar to those by PRIANISCHNIKOW, and found that the response of *Cucurbita* seedlings to ammonium salts was very different from that reported by PRIANISCHNIKOW (7, 8), mainly with respect to the accumulation of ammonia; however, the response of *Phaseolus* was similar to that reported (7, 8) for the starchy legumes.

It is unfortunate that PRIANISCHNIKOW did not grow his seedlings in complete nutrient solutions, as the distilled water and the solution of ammonium salts used unquestionably had effects which would not have appeared had the usual ions been present (15). There is evidence (10, 15) that calcium must play a more prominent rôle in altering the response of seedlings to ammonium salts than was realized by PRIANISCHNIKOW when he planned his experiments.

Employing improved methods, this investigation involves a further study of the effects of ammonium nutrition on the carbohydrate and nitrogen metabolism of etiolated seedlings as modified by the type and amount of food reserves in the seed. The present paper reports an attempt to throw additional light upon certain fundamental questions: How and to what extent do seedlings utilize ammonium nitrogen? What determines the rate of ammonium absorption? What conditions are associated with ammonium injury?

Materials and methods

Seeds of the following species were chosen: *Cucurbita pepo, Phaseolus vulgaris, Lupinus luteus,* and *L. albus.* The first three species are representative of PRIANISCHNIKOW'S three types respectively, while *L. albus* was selected as a possible intermediate between the second and third types. The composition of seeds of the various species as determined by SCHULZE (12) is partly given as follows (on the basis of dry weight of kernels): *Cucurbita* 33.07 per cent. protein, 55.20 per cent. ether extract; *Phaseolus* 24.06 per cent. protein, 43.98 per cent. starch; and *Lupinus luteus* 54.38 per cent. protein. SCHULZE and CASTORO (13) report *Lupinus albus* 43.06 per cent. protein.

After the usual precautions in the selection and germination of the seeds, young seedlings (3–4 cm. in length) were transferred to the culture solutions consisting of 0.006 M. $(NH_4)_2SO_4$, 0.0045 M. $MgSO_4 \cdot 7H_2O$, 0.0045 M. KH_2PO_4, and 0.0045 M. $CaCl_2$. A pH of 6.4 was obtained by addition of NaOH. The controls were made up similarly except that $(NH_4)_2SO_4$ was

omitted. The pH of the culture solutions was kept fairly constant by renewal at 48-hour intervals. The seedlings were grown in a dark room at 23°–25° C. In the first of the two series of experiments, the seedlings of the various species (except *Lupinus albus*) were grown in nutrient solutions for a period of ten days. In a second series the seedlings were grown until the ammonium seedlings showed injury.

The plant material was preserved and extracted with 80 per cent. alcohol. Nitrogen fractions were determined according to PHILLIPS *et al.* (**5**). After removing the alcohol from a portion of the alcoholic extracts, it was cleared and deleaded (**6**) before determining sugars. The bicarbonate modification of the Tompsett method as described by PHILLIPS (**4**) was employed in all carbohydrate determinations. Insoluble acid hydrolyzable polysaccharides and nitrogen were determined in the residue. Ether extract (**1**) was determined in the pumpkin seedlings.

Results

The results were calculated and studied on the basis of weight of constituents per hundred seedlings and are presented in part in table I. The results as expressed on other bases (**2**), not included here, may be calculated from data in table I. Conditions existing in the various species are more comparable when constituents are expressed as percentage fresh weight. Some correlations concerning ammonium absorption and utilization are presented in table II.

Discussion

Cucurbita pepo (*10 days*).—The increase in total nitrogen caused by ammonium was contributed largely by the ammonia fraction. The increase in amides was apparently due to a favorable carbohydrate-nitrogen balance; however, the increase was much less than that reported by PRIANISCHNIKOW (**7, 8**). Protein formation was favored by ammonium nutrition, but not at the expense of the protein reserves. Accumulation of reducing sugars in the stems and roots was remarkably checked by ammonium. Sucrose relations appeared to be somewhat obscure in this and other species studied.

The utilization of ether extract (oily reserve) by seedlings was somewhat retarded by ammonium nutrition, as shown by the following data (in percentage dry weight): Ten days: NH_4 cotyledons 5.40 per cent., control 4.88 per cent.; nineteen days: NH_4 cotyledons 4.49 per cent., control 3.67 per cent. The tendency to hinder utilization of the oily reserve was more pronounced in the 19-day ammonium injured seedlings. This injury was characterized by withering of the young leaves, which was also shown by ammonium injured *Phaseolus*. In all the species studied root discoloration was not associated with ammonium injury.

TABLE I

RESULTS OF CHEMICAL ANALYSES EXPRESSED AS WEIGHT PER HUNDRED SEEDLINGS

	Cucurbita 10 days		*Phaseolus* 10 days		*Phaseolus* 22 days		*Lupinus albus* 17 days		*Lupinus luteus* 9 days	
	NH_4	Control	NH_4	Control	NH_4	Control	NH_4	Control	NH_4	Control
Fresh weight	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Stems and roots	179.6	174.7	363.6	369.6	279.1	319.2	270.4	348.2	94.5	104.8
Cotyledons	44.1	40.3	14.0	14.6	3.1	3.0	81.0	80.5	56.8	57.9
Dry weight										
Stems and roots	5.80	5.96	22.33	22.16	16.92	18.77	13.22	14.02	4.59	5.06
Cotyledons	2.77	2.82	3.27	3.31	2.57	2.54	6.26	5.82	4.58	4.48
Total nitrogen	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Stems and roots	396.5	273.6	1709.1	1573.0	1690.0	1480.8	1612.3	1469.2	473.8	454.0
Cotyledons	194.1	174.1	77.2	81.5	73.5	60.9	626.5	561.8	546.9	538.4
Insoluble N										
Stems and roots	128.6	118.5	750.4	692.1	485.6	467.2	1128.2	1081.0	285.5	314.7
Cotyledons	139.4	122.9	59.2	60.5	53.0	43.5	443.0	392.9	311.5	306.2
Soluble N										
Stems and roots	267.9	155.1	958.7	880.9	1204.4	1013.6	484.1	388.2	188.3	139.3
Cotyledons	54.7	51.2	18.0	21.0	20.5	17.4	183.5	168.9	235.4	232.2
Amino N										
Stems and roots	132.0	112.4	423.8	401.7	361.3	325.2	205.6	190.8	91.0	86.6
Cotyledons	24.4	21.2	6.9	7.6	7.0	6.0	80.7	78.9	105.5	109.5
Amide N										
Stems and roots	21.1	15.8	173.8	166.3	171.6	171.2	81.0	45.4	34.3	37.4
Cotyledons	4.0	3.1	3.8	1.7	1.6	1.3	29.2	38.8	40.7	33.0
Ammonia N										
Stems and roots	84.6	14.1	33.0	18.0	133.0	37.2	102.0	14.8	35.7	6.2
Cotyledons	11.0	5.7	1.3	0.9	4.7	2.5	4.4	3.9	5.7	5.6
Reducing sugars										
Stems and roots	56.6	138.8	1290.4	1208.0	24.5	36.7	65.6	72.1	22.9	27.5
Cotyledons	63.8	34.7	41.1	48.1	18.7	19.6	31.7	29.0	28.9	30.4
Sucrose										
Stems and roots	54.1	35.2	225.6	262.4	4.4	18.0	25.3	30.4	21.4	19.0
Cotyledons	13.0	17.1	66.0	64.1	22.8	36.7	13.7	17.5	26.1	8.8

Phaseolus vulgaris (*10 days*).—Marked increases in ammonia and residual nitrogen resulted from ammonium nutrition. Although considerable amides accumulated, curiously, ammonium absorption did not materially increase this fraction due apparently to an unfavorable carbohydrate-nitrogen balance in which the high concentration of sugars associated with slow absorption of ammonium favored protein regeneration or synthesis. Ammonium absorption had practically no effect on the amide fraction in the ammonium injured seedlings (22 days). The conditions existing in these seedlings were somewhat analogous to those in the yellow lupines (9 days). An insufficient supply of sugars, even though much ammonia had accumulated, failed to result in amide synthesis, perhaps owing to lack of oxidation products of sugars. Considerable accumulation of residual nitrogen in both series suggests serious need of investigating this fraction.

On the 18th day, part of the cotyledons had fallen from the stems and at this time those still intact were removed and sampled. In both these and the 10-day cotyledons, ammonium tended to interfere with the utilization of starch as shown by the following data (mg. of starch in cotyledons per hundred seedlings): Ten days: NH_4 cotyledons 307 mg., control 293 mg.; eighteen days: NH_4 cotyledons 156 mg., control 144 mg. During the 10- to 18-day interval ammonium hindered utilization of reserve proteins.

Lupinus albus (*17 days*).—Ammonium injury was characterized by transparency followed by flaccidity of a region near the lower part of the hypocotyl. The roots of both series were apparently uninjured. Accumulation of amides in stems and roots of *L. albus*, which is favored by ammonium, distinctly differentiates these seedlings from injured *Phaseolus* (22 days). The conditions in white lupine with ammonium injury closely resembled those in yellow lupine with respect to the concentration of ammonia and amides. The higher concentration of reducing sugars associated with lower concentration of ammonia in ammonium injured *Lupinus albus* stems and roots as compared with ammonium injured *Phaseolus* indicated more favorable conditions for accumulation of amides in the former.

Lupinus luteus (*9 days*).—The type of ammonium injury especially peculiar to this species was characterized by a transverse rupture soon followed by discoloration and flaccidity of a region near the middle of the hypocotyl. In contrast to the white lupine, protein regeneration and formation of amides in stems and roots were hindered by ammonium. The increase of amides in cotyledons was associated with a higher concentration of reducing sugar and a lower concentration of ammonia than in stems and roots. Utilization of reserve protein was slightly hindered, while utilization and regeneration of insoluble acid hydrolyzable polysaccharides were seriously hindered by ammonium nutrition.

TABLE II

CORRELATIONS REGARDING RATES OF ABSORPTION AND UTILIZATION OF AMMONIUM NITROGEN (CALCULATED ON BASIS OF 100 GM. OF DRY KERNELS)

	NH_4-NITROGEN ABSORBED (A)	NH_4-NITROGEN ACCUMULATED (B)	ABSORBED NH_4-NITROGEN UTILIZED (A − B)	EFFICIENCY OF UTILIZATION (A − B)/(A) × 100
	gm.	*gm.*	*gm.*	%
Cucurbita pepo 10 days	1.551	0.873	0.678	43.7
Phaseolus vulgaris 10 days	0.357	0.041	0.316	88.6
Phaseolus vulgaris 22 days	0.642	0.257	0.385	59.8
Phaseolus vulgaris 10–22 days	0.285	0.216	0.069	24.2
Lupinus albus 17 days	0.730	0.308	0.422	57.8
Lupinus luteus 9 days	0.253	0.264	−0.011	.....

By correlating the results in table II with the composition of the kernels of the various species, it appeared that the nature and amount of the food reserves in the seed were important factors in determining the rate of ammonium absorption. The most rapid absorption was associated with *Cucurbita* (high oil reserve), while the lowest rate was associated with the high protein reserve of yellow lupine. Even though *Phaseolus* contained the lowest protein reserve, it did not favor rapid ammonium absorption. White lupine absorbed ammonium more rapidly than *Phaseolus* even though the former contained more protein reserve. Hence the nature of the non-nitrogenous food reserve appeared to be a decisive factor in governing ammonium absorption. Furthermore, the rate of absorption depended upon the stage of growth (state of nutrition) as shown by the 10-day and 10–22-day *Phaseolus*.

In the early stage of growth *Phaseolus* (10-day) accumulated but little ammonia through ammonium absorption, quite in contrast to *Cucurbita*. In the latter stage (10–22-days), as reserves were exhausted an amount of ammonia had accumulated comparable to that shown by *Lupinus luteus*. Five times as much ammonia accumulated as a result of ammonium absorption during the latter 12 days than during the first 10 days, owing to depletion of carbohydrate reserves.

The 17-day white lupine resembled 22-day *Phaseolus* in regard to efficiency of utilizing ammonium. In these seedlings, and especially in yel-

low lupine, ammonium nutrition seriously disrupted the formation and regeneration of organic nitrogen, this condition being associated with low non-nitrogenous food reserves.

Aside from or associated with the type and amount of food reserves, other peculiarities inherent in the various species may have altered the response of the seedlings to ammonium nutrition, and only through more extensive studies can certain generalizations be established.

Summary

1. Etiolated seedlings of *Cucurbita pepo, Phaseolus vulgaris, Lupinus albus,* and *L. luteus,* grown in complete nutrient solutions, were studied with respect to their response to ammonium nutrition as influenced by the type and amount of food reserves.

2. In this investigation the conditions which resulted during ammonium nutrition cannot be ascribed to physiological acidity. It is unfortunate that PRIANISCHNIKOW did not employ complete nutrient solutions.

3. The rates of absorption and utilization of ammonium per hundred grams of dry kernels were apparently dependent upon the type and amount of non-nitrogenous reserves and varied with the stage of growth. The efficiency of the various species with respect to the utilization of absorbed ammonium varied considerably.

4. Ammonium nutrition interfered with the utilization of reserve proteins and non-nitrogenous reserves. Protein regeneration or formation favored by ammonium did not take place at the expense of reserve proteins. Starved *Phaseolus* seedlings resembled those of *Lupinus luteus* in that protein regeneration was hindered by ammonium.

5. Little evidence was obtained in favor of the view emphasized by PRIANISCHNIKOW that amides serve as efficient detoxicants of ammonia.

6. The resistance of seedlings to ammonium injury was apparently governed by the type and amount of non-nitrogenous reserves in the seed. The type of ammonium injury was definitely different in the various species employed.

7. Considerable accumulation of ammonia and low concentration of reducing sugars were associated with ammonium injury which may or may not have been caused by these conditions. There is need of more extensive organo-chemical investigation supplemented with histological and physiochemical studies to determine more satisfactorily the conditions associated with ammonium injury.

8. Further investigation of these and other species is necessary to establish certain generalizations before attempting to classify seedlings with respect to their responses to ammonium salts.

The writer wishes to express his sincere appreciation to Dr. T. G. PHILLIPS whose continued interest and kind advice aided this investigation.

UNIVERSITY OF NEW HAMPSHIRE
DURHAM, NEW HAMPSHIRE

LITERATURE CITED

1. ASSOC. OFFIC. AGR. CHEMISTS. Official and tentative methods of analysis. 2nd ed. Washington. 1925.
2. BURKHART, L. The metabolism of etiolated seedlings as affected by ammonium salts. Thesis, Univ. New Hampshire. 1933.
3. HUNTER, H. M. The effects of ammonium chloride on the nitrogen metabolism of etiolated seedlings. Thesis, Univ. New Hampshire. 1932.
4. PHILLIPS, T. G. The determination of sugars in plant extracts. Jour. Biol. Chem. **95**: 735–742. 1932.
5. ————, *et al.* The determination of nitrogen in relatively simple compounds. Plant Physiol. **2**: 205–211. 1927.
6. POTTER, G. F., and PHILLIPS, T. G. Composition and fruit bud formation in non-bearing spurs of the Baldwin apple. New Hampshire Agr. Exp. Sta. Tech. Bull. 42. 1930.
7. PRIANISCHNIKOW, D. N. The rôle of ammonia in the metabolism of nitrogenous substances in plants. Intl. Rev. Sci. & Pract. Agr. **8**: 204–216. 1917.
8. ————. Das Ammoniak als Anfangs- und Endprodukt des Stickstoffumsatzes in den Pflanzen. Landw. Vers.-Sta. **99**: 267–286. 1922.
9. ————. Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. deutsch. bot. Ges. **40**: 242–248. 1922.
10. ————. Zur Frage über die Bedeutung des Calciums für die Pflanzen. Ber. deutsch. bot. Ges. **41**: 138–144. 1923.
11. ————, and SCHULOW, J. Über die synthetische Asparaginbildung in den Pflanzen. Ber. deutsch. bot. Ges. **28**: 253–264. 1910.
12. SCHULZE, E. Über die chemische Zusammensetzung der Samen unserer Kulturpflanzen. Landw. Vers.-Sta. **73**: 35–170. 1910.
13. ————, and CASTORO, N. Beiträge zur Kenntnis der Zusammensetzung und des Stoffwechsels der Keimpflanzen. Zeitschr. physiol. Chem. **38**: 199–258. 1903.
14. SMIRNOW, A. I. Über die Synthese der Säureamide in den Pflanzen bei Ernährung mit Ammoniaksalzen. Biochem. Zeitschr. **137**: 1–34. 1923.
15. TRUE, R. H. The function of calcium in the nutrition of seedlings. Jour. Amer. Soc. Agr. **13**: 91–107. 1921.

PRODUCTION OF ALCOHOL AND ACETALDEHYDE BY TOMATOES[1]

FELIX G. GUSTAFSON

Introduction

Ever since the time of PASTEUR it has been known that under some conditions seed plants produce small quantities of alcohol. LECHARTIER and BELLAMY (**10**) were the first to show that fruits, when deprived of air, produce alcohol as well as CO_2. These workers studied a number of varieties of fruits and always found some alcohol produced. They inclosed their fruits in tight containers for several months and in many cases the fruits were considerably disintegrated although they were sterile. From his extensive studies on the maturing of fleshy fruits, GERBER (**3**) came to the conclusion that in many fruits alcohol is formed during the process of ripening, together with volatile acids and esters. MÜLLER-THURGAU and OSTERWALDER (**13**) found alcohol in pears. THOMAS (**18**) found alcohol in apples. Although GERBER studied apples he does not mention having found alcohol in these fruits. ONSLOW and BARKER (**17**) found alcohol in oranges. The concentration was particularly high when the fruits had been stored in an atmosphere high in CO_2, but even in ordinary air alcohol was present to the extent of about 0.03 to 0.10 per cent.

It is thus evident that alcohol is produced in fruits when they are surrounded by air. Under anaerobic conditions alcohol has been found in many plant structures, and reference to much literature on the subject is to be found in the monographs (**8**) on plant respiration.

It has been shown by a number of investigators that acetaldehyde is formed in plants. MAZÉ (**11**) found acetaldehyde in unripe seeds of corn and pear. This acetaldehyde he believed prevented them from germinating. In 1912 KOSTYTSCHEW (**7**) demonstrated that acetaldehyde was produced in the alcoholic fermentation of yeast. His method of demonstrating acetaldehyde consisted in adding zinc chloride to the fermenting liquid, which brought about polymerization of the acetaldehyde, preventing it from being reduced to alcohol. KOSTYTSCHEW *et al.* (**9**) found acetaldehyde in poplar blossoms. Acetaldehyde has also been found in other plants: by MÜLLER-THURGAU and OSTERWALDER (**13**) in pears and apples; by NEUBERG and REINFURTH (**14**) in alcoholic fermentation; by NEUBERG and GOTTSCHALK (**15**) in crushed peas and bananas; by THOMAS (**18**) in apples; by BODNAR and coworkers (**1**) in whole seeds of peas; by KLEIN and PIRSCHLE (**6**) in flowers, leaves, seedlings, and seeds of a number of different species of plants; by HARLEY and FISHER (**5**) in ripe pears.

[1] Paper from the Department of Botany of the University of Michigan, no. 400.

That acetaldehyde is an intermediate compound in anaerobic respiration seems to have been first shown by KOSTYTSCHEW (7), although NEUBERG is given all of the credit. He certainly demonstrated beyond a doubt that acetaldehyde is one of the intermediate products formed during alcoholic fermentation. NEUBERG and REINFURTH by their "Abfangsmethode" have been able to show that acetaldehyde is produced in large quantities during alcoholic fermentation. Their first method consisted of adding sodium bisulphite to the fermenting liquid; the bisulphite united with the acetaldehyde and thus prevented it from being reduced to alcohol. In 1920 they published a second method of binding the acetaldehyde. In this method one molecule of acetaldehyde united with two molecules of dimethylhydroresorcin (dimedon). After the fermentation had been completed the acetaldehyde was quantitatively determined.

Investigation

The work reported in this paper was undertaken as a further investigation of respiration in tomato fruits. The fruits used were picked from the vines as needed, thus insuring freshness of the material.

Both alcohol and acetaldehyde were determined in the fruits and leaves as they came from the plants and also after the fruits had been respiring anaerobically for various lengths of time. Nitrogen was used in most of the experiments to replace air, although a few experiments were conducted in which CO_2 replaced air. In four experiments the fruits were placed in a large container, which was then closed, and as respiration proceeded the oxygen was used up and replaced with CO_2.

When either CO_2 or nitrogen was used to replace the air, these gases were passed through wash bottles containing chromous chloride, to remove all oxygen. The gas was then passed through the chamber containing the fruits. In the experiments on alcohol production fruits were in a nitrogen atmosphere for 24, 48, and 72 hours. For these experiments a quantity of fruits was collected and divided into four groups, each group having fruits of the same ripeness and size. Only uninjured fruits were used. One lot of fruits was analyzed at once, and the others were placed in separate containers through which nitrogen was passed. One lot was analyzed each day. In this way it was possible to study the progressive accumulation of alcohol in similar fruits.

Several methods of determining alcohol were tried. In the beginning a method of steam distillation was used. A weighed quantity was taken and the fruits cut into small pieces, placed in a 5-liter round-bottom flask and steam-distilled for 5 to 6 hours, or until about 600 cc. of distillate had been collected. The distillate was collected in two receiver flasks, each containing 50 cc. distilled water at the beginning, and surrounded by ice. The

total distillate was made up to 760 cc. each time by addition of distilled water, and 500 cc. of this were used for the alcohol determination and 260 cc. for the acetaldehyde determination.

Acetaldehyde was determined according to the method of NEUBERG and GOTTSCHALK (16). For the acetaldehyde determination 260 cc. of the distillate were used. To this 40 cc. of hydroxylamine sulphate (1.0 per cent.) were added and the mixture permitted to stand for an hour. The H_2SO_4 liberated was titrated with 0.1 N NaOH, using methyl orange as indicator. To insure that the same end point was always reached, two buffer flasks with pH 3.6 and 5.8 were used, each having the same amount of liquid as the titration flask. A correction was made for the neutralizing effect of distilled water. From several titrations of laboratory distilled water it was found that the addition of 100 cc. of distilled water was equivalent to 0.2 cc. 0.1 N NaOH. NEUBERG and GOTTSCHALK give the conversion factor as 1 cc. 0.1 N NaOH equivalent to 0.0044 gm. acetaldehyde.

The alcohol and acetaldehyde were oxidized to acetic acid with potassium dichromate and sulphuric acid. This was then distilled from a claison flask and the acetic acid titrated with 0.1 N NaOH, using phenolphthalein as indicator. By calculation the total alcohol in the 760 cc. of distillate was found. The acetic acid formed from the aldehyde was of course subtracted from the total in making this calculation. This method of determining alcohol gave very high values, and as other methods gave only about half as high a percentage these figures are not presented. The high concentration of alcohol is probably due to the long distillation at steam temperature. This probably distilled over some other substances, such as esters, in addition to alcohol and acetaldehyde, which later reacted with the NaOH in the titration.

If this method of the distillation vitiated the alcohol determination it did the same to the acetaldehyde analysis. The acetaldehyde analyses are not presented because of their lack of reliability. All one can say is that according to the determinations following the NEUBERG-GOTTSCHALK method, acetaldehyde was always found to be present. How much of this apparent acetaldehyde was due to acids that may have distilled over is not known. There was very little variation in the acetaldehyde noted, and there was no accumulation as the time of anaerobiosis was continued. The second method of alcohol determination did not lend itself readily to acetaldehyde analysis and it was therefore not repeated.

The alcohol was finally determined according to the method used by CANNAN and SULZER (2) in determining alcohol in blood. The apparatus used was modified to utilize more material than these investigators used. In these experiments a known amount (about 300 gm.) of freshly ground tomato pulp was placed in a 2-liter round-bottom flask, and between 500

and 600 gm. of anhydrous Na_2SO_4 added to prevent frothing. Distillation was carried out at a reduced pressure, which was around 6 cm. of mercury for all experiments, into two tubes 2 × 25 cm., each containing 35 cc. of concentrated H_2SO_4. At the pressure of 6 cm. mercury bubbling or boiling was produced at 40° C. In no experiment did the temperature rise as high as 45° C. After several preliminary experiments the time of distillation was placed at three hours. The H_2SO_4 from both receiving tubes was brought together and the volume made up to 100 cc. A 20-cc. portion of this H_2SO_4 was added drop by drop to a known quantity of 0.2 N $K_2Cr_2O_7$, which quantity varied with the experiment. The dichromate flask was kept cool by immersion in running water while the sulphuric acid was added. The oxidizing mixture of sulphuric acid and potassium dichromate was allowed to stand for one hour to complete the oxidation of the alcohol to acetic acid. This solution was then diluted until the sulphuric acid was 5 per cent., and an excess of 10 per cent. KI added (about 10 cc.). The iodine liberated by the excess dichromate was titrated with 0.1 N sodium thiosulphate, using starch as the indicator. In this determination 1 cc. 0.1 N $K_2Cr_2O_7$ is equivalent to 1.15 mg. of alcohol.

With the CANNAN-SULZER method much more complete and better organized experiments were conducted than with the first mentioned method. For this reason, and also because it is a more reliable method, the results obtained with it are presented in table I.

Table I shows that there is considerable alcohol in the fruits that have been in air; that this is at its maximum in the nearly ripe orange-red fruits; and that in the green fruits the amount of alcohol decreases as the fruit size is decreased, even though the small young fruits respire very much more than the larger fruits. During these investigations an experiment was conducted having as its purpose to study the relation between size and alcohol production. For this experiment orange colored tomato fruits were chosen, because among the ripening fruits they respire rapidly and for the experiment it was necessary to have fruits of the same physiological age. In one lot the fruits were 10.0 cm. in diameter and in the other lot only 3.5 cm. in diameter. Analysis was made directly after the fruits were removed from the vines. The large fruits had an alcohol content of 0.012 per cent. while in the smaller fruits only 0.0081 per cent. alcohol was found. From this experiment as well as from the experiments noted in table I it seems evident that when respiration is rapid the alcohol formation (*i.e.*, the anaerobic respiration) is greater in the large fruits than in the smaller ones.

Table I also shows that alcohol, unlike acetaldehyde, accumulates as the time of anaerobiosis lengthens; and that the alcohol increase is greatest in those fruits carrying on the most rapid respiration, namely, the small green fruits, in which the initial alcohol content was by far the smallest. There

TABLE I

PERCENTAGE OF ALCOHOL IN TOMATO FRUITS

CONDITION OF FRUITS	DIRECTLY FROM PLANT	NUMBER OF HOURS IN NITROGEN		
		24	48	72
	%	%	%	%
Red-ripe	0.0125	0.04	0.065	0.071
	0.0140	0.031	0.057	
	(1)*0.0144	(1)0.031	(1)0.054	(1)0.070
			0.054	
Orange-red	0.023	(2)0.0255	(2)0.0555	(2)0.0315
	(3)0.025	(3)0.028	(3)0.061	(3)0.046
Orange	(4)0.016	(4)0.037	(4)0.040	(4)0.062
Pink-orange			0.041	
			0.035	
Pink	0.012	(5)0.016	(5)0.034	
	(6)0.016	(6)0.017	(6)0.096†	(6)0.042
Green Large	0.016	(7)0.021	(7)0.047	(7)0.094
	0.014			
Medium	(8)0.012	(8)0.039	(8)0.077	(8)0.133
Small	(9)0.008	(9)0.066	(9)0.116	(9)0.150
2 cm. diameter	(10)0.0015	(10)0.003	(10)0.095	(10)0.185
1–2 cm. "	0.0011	(11)0.104	(11)0.135	(11)0.205

* Similar numbers in the brackets before the figures indicate that these fruits were picked at the same time and were all alike, but have been in nitrogen different lengths of time.

† Fruit very soft when taken out of nitrogen.

is one exception to the preceding statement. In the orange-red fruits there is a decrease in amount of alcohol from 48 hours to 72 hours. There is no apparent reason for this decrease.

TABLE II

PERCENTAGE OF ALCOHOL IN OTHER PLANT STRUCTURES

MATERIAL	IN AIR	IN NITROGEN 48 HOURS
Potato Old	0.0013	0.019
New	0.0012	
Peas germinated 48 hours	0.037	0.244
Tomato leaves	0.0031	..

It should be pointed out that although table I is headed alcohol yet it actually applies to alcohol plus acetaldehyde. Both are oxidized by the sulphuric acid and the potassium dichromate. The amount of acetaldehyde, however, is a very small part of the total volatile material as given in table I.

Table II presents a miscellaneous collection and is presented only for the sake of comparison. It is usually stated in works on respiration (8) that potatoes do not produce any alcohol, and that germinating peas produce large quantities of it. From table II one must conclude that some alcohol is produced in potatoes although in small quantity, and that this quantity increases only very slightly when the tubers are completely deprived of oxygen for 48 hours. Peas germinating in air produce somewhat more alcohol than orange-red tomatoes, and the amount of alcohol increases very much when the peas are deprived of oxygen for a period of 48 hours. The alcohol content in tomato leaves directly from the vines is intermediate between that from tomato fruits with a diameter of 2 cm. and that from fruits with a diameter of 4 to 5 cm.

Determination of acetaldehyde depends upon titration with a base, and it is obvious that any acid which had distilled over would increase the amount of hydroxide needed to bring about neutralization and this would increase the apparent amount of acetaldehyde. Also other alcohols than ethyl alcohol would be oxidized by the H_2SO_4 and $K_2Cr_2O_7$ and the apparent amount of ethyl alcohol would thereby be increased. Therefore it was deemed appropriate to make qualitative tests for acids, other alcohols, and aldehydes to discover whether the figures just cited could be considered to represent the ethyl alcohol as stated in the tables.

Not only acids and alcohols were tested for but also other compounds with a boiling point near 100° C. and known to be associated with anaerobic respiration. These compounds were: formic and acetic acids, formaldehyde, acetaldehyde and higher aldehydes, methyl, ethyl, n. propyl, isopropyl, isoamyl and isobutyl alcohols, acetone, acrolein, and methyl glyoxal. Of these acetaldehyde, ethyl alcohol, and a trace of acids were always found. In several experiments there was a trace of methyl alcohol. In one experiment each formic and acetic acids and formaldehyde were found as a trace. The other compounds were always absent. The experimental material consisted of fresh leaves, large green fruits, also small green, large green, faintly pink or yellow, and red ripe fruits, which had been respiring anaerobically from 48 to 96 hours in nitrogen. This material includes all that was used in the quantitative analyses.

From the results of these qualitative analyses it seems fairly certain that the only aldehyde found extensively is acetaldehyde, and only ethyl alcohol is formed; and it can then be concluded that in the respiration of tomatoes

(both when in air and when deprived of oxygen) acetaldehyde and alcohol are formed. Since acetaldehyde does not accumulate as the time of anaerobiosis lengthens, it is evident that it is not a final product of anaerobic respiration. On the other hand alcohol does accumulate and is therefore a final product of anaerobiosis.

The experiments bring out the fact that in the larger fruits there is a certain amount of anaerobic respiration even when the fruits are surrounded by air. In a previous paper the writer (**4**) pointed out that, from the behavior of the fruits when deprived of oxygen, it seemed very likely that there was some anaerobic respiration in tomato fruits even when in air, and these experiments prove that this assumption was correct. The experiments further show that the anaerobic respiration when the fruits are in air is much greater in large than in small fruits of the same physiological age. At least the alcoholic formation is greater in the large fruits than in the small ones, when both fruits are orange in color.

As yet no analyses have been made of the gas from the interior of tomato fruits, but the CO_2 content is probably high and the oxygen low. This is to be expected from other work, as that of MAGNESS (**12**) on apples and potatoes and some unpublished analyses of the gas from cacti by the writer. According to KOSTYTSCHEW and others, the preliminary steps in all types of respiration are the same. If that is true there is no reason why the aerobic and anaerobic respirations should not take place at the same time in a fruit, when the oxygen concentration becomes low. Some molecules of the intermediate compounds may follow the aerobic respiration path leading to the CO_2 and H_2O formation, while others may be changed to CO_2 and C_2H_5OH. The ratio between the two types of respiration would vary with the amount of oxygen present. As the oxygen became depleted the anaerobic type would increase, until finally all of the respiration was of that kind.

In his work on apples, THOMAS (**18**) distinguishes two types of respiration in which alcohol is formed. In the absence of oxygen there is little acetaldehyde formed with the alcohol. This type he calls anaerobic zymasis. The other type which he calls carbon dioxide-zymasis takes place in a high concentration of CO_2 in the presence of oxygen. Under this condition a high percentage of acetaldehyde is formed together with the alcohol. In the experiments on tomatoes there is no such distinction. THOMAS reports that in carbon dioxide-zymasis the acetaldehyde alcohol ratio was near one to two. There is no essential difference in this ratio in the absence of oxygen and in its presence, in the work of the tomato fruits. The writer's experiments on carbon dioxide-zymasis were not so extensive as those of THOMAS, but nevertheless it seems that there is a decided difference in the behavior of apples and tomatoes.

Summary

1. Acetaldehyde has been found in all tomato fruits, under all conditions of treatment. The amount of this aldehyde does not increase as the time of anaerobiosis lengthens.

2. Ethyl alcohol has also been found in all tomato fruits, under all conditions of treatment. The amount of alcohol increases with the length of anaerobiosis.

3. Fruits as taken off the vines, which had been in the greenhouse under natural conditions, contained some acetaldehyde and alcohol, although not so much as in fruits deprived of oxygen.

4. Large fruits contained more alcohol than small fruits when analyzed directly from the vines.

5. In qualitative experiments, compounds known to be associated with anaerobic respiration and having a boiling point near 100° C. were tested for. Acetaldehyde and ethyl alcohol were always found. Traces of methyl alcohol were found in several experiments, and traces of formic and acetic acids and formaldehyde were each found in only one experiment.

Acknowledgment is made of a grant from the Faculty Research Fund of the University of Michigan for most of the analytical work, which was done by Miss Edna Warweg and Mr. Sam Granick.

University of Michigan
Ann Arbor, Michigan

LITERATURE CITED

1. Bodnar, J., Szepessy, Charlotte, and Ferenczy, Johann. Die Anwendung der Neubergschen Acetaldehyd-Abfangmethode bei der alkoholischen Gärung höherer Pflanzen. Biochem. Zeitschr. **165** 16–22. 1925.
2. Cannan, R. K., and Sulzer, R. The estimation of alcohol in blood. Heart **11**: 148–150. 1924.
3. Gerber, M. C. Recherches sur la maturation des fruits charnus. Ann. Sci. Nat. Bot. VIII. **4**: 1–279. 1896.
4. Gustafson, F. G. Intramolecular respiration of tomato fruits. Amer. Jour. Bot. **17**: 1011–1027. 1930.
5. Harley, C. P., and Fisher, D. F. The occurrence of acetaldehyde in Bartlett pears and its relation to pear scald and breakdown. Jour. Agr. Res. **35**: 983–993. 1927.
6. Klein, G., and Pirschle, K. Acetaldehyd als Zwischenprodukt der Pflanzenatmung. Biochem. Zeitschr. **168**: 340–360. 1926.
7. Kostytschew, S. Über Alkoholgärung. I. Über die Bildung von

Acetaldehyd bei der alkoholischen Zuckergärung. Zeitschr. physiol. Chem. **79**: 130–145. 1912.

8. ————. Plant respiration. Transl. by C. J. LYONS. P. Blakiston's Son and Co., Philadelphia. 1927.
9. ————, HÜBBENET, E., and SCHELOUMOFF, A. Über die Bildung von Acetaldehyd bei der anaeroben Atmung der Pappelblüten. Zeitschr. physiol. Chem. **83**: 105–111. 1913.
10. LECHARTIER, G., and BELLAMY, F. De la fermentation des fruits. Compt. rend. Acad. Sci. (Paris). **69**: 466–469, 1869; **79**: 1006–1009; 1874.
11. MAZÉ, P. Maturation provoquee des graines. Action antigerminative de l'aldéhyde éthylique. Compt. Rend. Acad. Sci. (Paris). **151**: 1383–1386. 1910.
12. MAGNESS, J. R. Composition of gases in intercellular spaces of apples and potatoes. Bot. Gaz. **70**: 308–316. 1920.
13. MÜLLER-THURGAU, H., and OSTERWALDER, A. Acetaldehydbildung in Obstfrüchten. Landwirt. Jahrb. Schweiz. **29**: 397. 1915.
14. NEUBERG, C., and REINFURTH, E. Die Festlegung der Aldehydstufe bei der alkoholischen Gärung. Biochem. Zeitschr. **89**: 365–414. 1918.
15. ————, and GOTTSCHALK, A. Beobachtungen über den Verlauf der anaeroben Pflanzenatmung. Biochem. Zeitschr. **151**: 167–176. 1924.
16. ————, and ————. Quantitative Untersuchungen über die Bildung und Herkunft des Acetaldehyds im intermediären Zellstoffwechsel der Warmblüter. Biochem. Zeitschr. **146**: 164–184. 1924.
17. ONSLOW, M., and BARKER, J. The alcohol content of oranges and its diagnostic value. Report Food Investigation Board. 1927. p. 35.
18. THOMAS, M. A quantitative study of the production of ethyl alcohol and acetaldehyde by cells of the higher plants in relation to concentration of oxygen and carbon dioxide. Biochem. Jour. **19**: 927–947. 1925.

COMPARISON OF THE HEATING AND FREEZING METHODS OF KILLING PLANT MATERIAL FOR CRYOSCOPIC DETERMINATIONS

T. D. MALLERY

In a study of the changes in the sap concentration of *Larrea tridentata* reported by the writer (1), the leaf and twig tissues were killed by heating them in closed vessels in boiling water for a period of 30 minutes before the sap was extracted for the cryoscopic determinations. This is a new departure in cryoscopic studies in this country, where plant tissues have been killed by subjecting them to freezing temperatures. The heating method, however, has been used extensively by WALTER (3). The method is described in detail in the paper just cited (1).

On theoretical grounds the heating method of rendering plant tissues permeable to the cell contents is open to criticism. Both enzymatic changes and other chemical reactions may be speeded up two to three times for every 10° C. rise in temperature; that is, the hydrolysis of sugars and other cell contents may occur which would change the osmotic concentration of the cell sap from its natural condition. In order to establish the practical importance of such changes in relation to a determination of the Ov of any particular species, comparative studies were made between the heating and freezing methods. The purpose of this paper is to report the results of these experiments.

In cooperation with Dr. MATLOCK and Mr. HOBART of the agronomy department of the University of Arizona, a comparative study was conducted using field cotton. This experiment also served the purpose of comparing results obtained by the use of a Beckmann thermometer with those found when a Drucker-Burian thermometer was employed. The material used for this experiment was cotton grown on experimental plots on the university farm near Tucson. Two sets of leaf samples were collected simultaneously from each of two plots. Each plot was divided roughly into four sections, each set of samples consisting of one sample from each section and each sample containing about twelve leaves picked at random from plants within a given section. One set of samples from each plot was collected by MATLOCK and HOBART, each collecting two samples, while the other two sets were collected by the writer. One set of samples from each plot was frozen in an ice-salt mixture and one set from each plot was heated in boiling water for 30 minutes. All of the osmotic value determinations for plot no. 7 were made by MATLOCK and HOBART, using a Beckmann thermometer, and they also made the determinations for the set of samples which they collected on plot no. 6. The osmotic values for the other set of samples

from plot no. 6 were determined by the writer, using a Drucker-Burian thermometer. The results of this experiment are given in table I.

TABLE I

COMPARISON OF THE FREEZING AND BOILING METHODS OF KILLING COTTON LEAF TISSUES FOR SAP EXTRACTION IN CRYOSCOPIC DETERMINATIONS

PLOT	SECTION	FROZEN Δ	HEATED Δ	FROZEN Ov	HEATED Ov	Δ OF HEATED − Δ OF FROZEN MATERIAL	Ov OF DIFFERENCE
7	1	0.940	1.120	11.320	13.480	0.180	2.171
	2	1.016	1.102	12.232	13.264	0.086	1.037
	3	0.934	0.992	11.248	11.944	0.058	0.699
	4	1.133	1.201	13.636	14.452	0.068	0.820
Average	.....	1.006	1.104	.....	.....	0.097	1.181
6	1	1.056	1.111	12.712	13.372	0.055	0.663
	2	1.395	1.007	16.780	12.124	− 0.338	4.676
	3	0.848	1.047	10.216	12.604	0.199	2.399
	4	1.562	1.313	18.784	15.796	− 0.249	3.001
Average	.....	1.215	1.120	.....	.....	− 0.223	2.688

It will be noted that for plot no. 7, all determinations for which were made with a Beckmann thermometer by the same operators, the heated material yielded a higher osmotic value in each case than did the frozen material. This fact would tend to support the theory that hydrolysis or enzymatic action took place in the heated material. However, the difference between the depressions of the freezing point found in the frozen material for sections 3 and 4 of plot no. 7 is 0.199°, which is equal to 2.399 atmospheres, and this could be taken as an indication that differences in the values are attributable to variations in the material and lack of uniformity in the sampling process. On the other hand, this is somewhat offset by the fact that the differences between sections 3 and 4 of plot no. 7 for the heated material are practically the same and are in the same order as those for the frozen tissue.

When the results for plot no. 6 are compared, greater differences and much less uniformity are apparent. This is somewhat to be expected, since the determinations were made by different operators using different type thermometers. However, in this case the values found for the heated material are not uniformly higher than those of the frozen material. Such varia-

tions cannot be ascribed entirely to the method of killing, since changes brought about by raising the temperature of the tissues would, in the majority of plant tissues at least, increase the osmotic concentration of the sap. The difference between the highest and lowest values for the frozen material is a little over 0.7° C., while the highest and lowest values for the heated material show a difference of a little over 0.3° C. Again these facts would tend to show that differences between the values for the frozen and heated material are due to actual differences in the samples, and not to the method of killing or of determining the depressions of the freezing point. It would seem, therefore, that the results of this experiment are not against the use of the heating method of killing tissue for juice extraction in cryoscopic determinations, even with a succulent, rapidly growing plant like cotton, with large leaves in which the amount of hydrolyzable material is probably much greater than in the more slowly growing, non-succulent, small leaved *Larrea.*

WALTER (3) presents some comparative data on values obtained for living tissue (L), tissue killed by heating in water at 100° C. (H), and tissue killed by freezing with liquid nitrogen (K). Several species of plants were used. For leaves of *Vitis* he found the following values: L = 7.47, H = 10.48, K = 10.00 atmospheres; for *Parictana:* L = 7.47, H = 10.61, K = 11.25 atmospheres. The sap expressed from *Buxus sempervirens,* a thick heavy leaved plant, gave values: L = 7.16, H = 25.64 and 25.49, and K = 25.95 and 26.74 atmospheres. In the case of *Aucuba japonica* the method of killing the tissue seems not to be important, for three samples of leaf tissue killed by heating at 100° C. gave osmotic values for their expressed sap of 21.28, 20.50, and 21.08 atmospheres (average = 20.96 atms.), while three samples killed by freezing with liquid nitrogen gave values of 21.94, 22.00, and 19.77 atmospheres (average = 21.30). *Ficus elastica* also gave values which were very close for heated and frozen material, the values for the former being 9.73 and 9.68 atmospheres and those for the latter 9.90 and 9.67 atmospheres.

Determinations of the osmotic values of small whole plants taken from their natural environment showed, in the work of WALTER, no distinct influence of the method used in killing the tissues before expressing the sap. The results of this experiment were as follows:

Thymus serpyllum	H = 12.03	and	12.56	atmospheres
	K = 10.23	"	13.02	"
Allyssum montanum	H = 14.00	"	14.84	"
	K = 14.10	"	12.64	"
Pirola umbellata	H = 21.09	"	K = 19.86	"

Experiments with *Prunus laurocerasus* resulted in increasingly higher osmotic values for the cell sap as the period of killing the tissues by heating

was lengthened, and when the heating was continued long enough completely to kill all of the cells, H = 25.09 and 25.13 atmospheres, as compared with the values for K which were 22.36 and 22.26 atmospheres. These results were attributed to the fact that *Prunus* contains relatively large quantities of easily hydrolyzable glucosides, which upon hydrolysis increase the osmotic concentration of the cell sap. When working with such plants or plant tissue, if very exact determinations are required the method of killing by freezing should be employed.

Further to test the reliability of the heating method, a comparison with the freezing method was made using leaves and small twigs of *Larrea tridentata*. For this experiment samples of leaves and twigs were collected from *Larrea* bushes near the main laboratory building. The first collection consisted of twelve samples from bush A, eight from bush B, and four each from bushes C, D, E, and F. The second collection, made 4 days later, included four samples each from the same bushes except bush D and from three additional bushes, G, H, and I. The samples were all taken from the bushes in rapid succession, and immediately the samples from each bush were divided into two lots. One of these was subjected to the heating method of killing, the other to the freezing method.

The freezing of the plant tissues was accomplished with solid carbon dioxide (dry ice), which gives a temperature of about −180° C. The samples were in glass test tube-like vessels with cork stoppers which fit compactly, in aluminum canisters with screw tops. These canisters were completely buried in dry ice in a calorimeter tank, which in turn was lowered into a 5-gallon crock and completely covered with towels. The samples from the first collection were left in the freezing chamber 50 minutes, 30 minutes, and 12 hours, as indicated in table II, which also gives the osmotic values obtained for these samples.

In eleven of the eighteen comparisons shown in table II, the values for the heated plant materials were greater ($>$) than those found for the frozen tissues. In five instances the compared values were equal or nearly so, and the samples from bush E gave values for the heated materials which were less ($<$) than those for the frozen samples, although the differences were less than one atmosphere.

These results lend some support to the possibility that the heating process may produce significant changes in the concentration of the plant sap. In only three instances, however, was the difference between the values for the heated and frozen material greater than two atmospheres, and in but eight of the eighteen comparisons was the difference more than one atmosphere. While these differences are greater than desirable, they are partly attributable to chance pairing. For example, in the case of bush B, samples 22 and 23, the difference is 3.05 atmospheres. If sample

TABLE II

OSMOTIC VALUES OBTAINED FOR COMPARABLE SAMPLES (FIRST COLLECTION) OF LEAVES AND TWIGS OF *LARREA TRIDENTATA* BY HEATING AND BY FREEZING METHODS OF KILLING THE TISSUES PRIOR TO EXPRESSING SAP

BUSH	HEATED TISSUES			COMPARISON OF Ov's	FROZEN TISSUES			DIFFERENCE IN Ov's
	SAMPLE NO.	Ov	DURATION OF TREATMENT		SAMPLE NO.	Ov	DURATION OF TREATMENT	
			min.				*min.*	
A	12	28.17	30	>	13	26.37	50	1.80
"	14	29.06	"	>	15	26.55	"	2.51
"	16	28.11	"	>	17	27.03	"	1.08
"	18	27.75	"	>	19	27.11	"	0.64
B	20	30.20	"	=	21	30.26	"	0.06
"	22	31.99	"	>	23	28.94	"	3.05
"	24	29.90	"	=	25	29.90	"	0.00
"	26	30.98	"	>	27	28.86	"	2.12
C	28	28.94	"	=	29	28.70	30	0.24
"	30	29.30	"	>	31	28.29	"	1.01
D	32	41.12	"	>	33	39.57	"	1.55
"	34	40.76	"	>	35	40.05	"	0.71
E	36	34.83	"	<	37	35.75	"	0.92
"	38	33.78	"	<	39	34.56	"	0.78
F	40	28.58	"	=	41	28.58	"	0.00
"	42	27.93	"	=	42	28.11	"	0.18
							hr.	
A	38×	26.49	"	>	37×	25.05	12	1.44
"	40×	26.55	"	>	39×	25.11	"	1.44

22 had been paired with sample 21 the difference would have been only 1.73 atmospheres. It will be noticed also that some of the heated samples, 22 and 24 for example, exhibited as great differences as 2.09 atmospheres.

The average of the first four values obtained for the heated material from bush A is 28.25 atmospheres and for the frozen material 26.76 atmospheres. The difference between these average values is but 1.49 atmospheres. The average value for the heated tissues from bush B is 30.77 atmospheres and for the frozen samples from B, 29.49 atmospheres, making a difference of only 1.25 atmospheres. Using the differences between the average osmotic values found for bushes A and B for comparison, instead of the paired sample differences, leaves only one case in the entire first collection in which the heated tissues yielded a value more than 1.50 atmospheres higher than that for the frozen material.

Previous investigations with *Larrea* have shown that a difference of as much as 0.5 atmospheres may be attributed to variations in the samples

from the same bush. If this amount be subtracted from the comparative difference, only about 1 atmosphere increase in the osmotic value of the sap need be attributed to the heating process.

TABLE III

OSMOTIC VALUES OBTAINED FOR *LARREA* FROM THE SECOND COLLECTION. FOURTEEN SAMPLES WERE HEATED 30 MINUTES, TEN WERE FROZEN 12 HOURS, AND FOUR WERE UNTREATED PRIOR TO EXTRACTING THE SAP

BUSH	HEATED TISSUES		COMPARISON OF Ov'S	FROZEN TISSUES		UNTREATED TISSUES		DIFFERENCES IN Ov'S
	SAMPLE NO.	Ov		SAMPLE NO.	Ov	SAMPLE NO.	Ov	
A	1×	24.10	<			2×	27.57	3.47
"	7	23.74	<	9	26.67			2.93
B	10	29.00	<			28	31.27	2.27
"	29	29.48	<	30	29.90			0.42
C	48	28.05	<			5	32.05	4.00
"	35	27.27	<	36	30.54			3.27
E	20	23.38	<			21	25.77	2.39
"	22	23.38	<	23	27.15			3.77
G	11	38.79	<	17	42.31			3.52
"	18	38.56	<	19	43.68			5.12
H	31	27.09	<	32	30.98			3.89
"	33	27.69	<	34	31.51			3.82
I	37	27.63	<	38	29.48			1.85
"	39	27.69	<	40	28.17			0.48

The data presented in table III for the second collection of *Larrea* leaves and twigs completely reverse the evidence in regard to the heating and freezing method of killing plant material for cryoscopic determinations of its sap concentration. In this series of determinations the heated samples gave osmotic values which were, in every case, less than those found for the frozen and the untreated tissues.

This astonishing difference in results can probably be attributed almost entirely to the fact that, while the determinations of the Ov for the first collection were made the following day, it was necessary to store the second collection of samples nearly three weeks before the osmotic values were determined. The freezing process did not sterilize the samples as did the heating method, and apparently the storage temperature was not sufficiently low to prevent bacterial and enzymatic activity. The fact that the values for the untreated material more nearly equal the values for the frozen samples than those for the heated tissues, and are in most cases higher than the frozen, helps to bear out the preceding contention.

Summary

1. A comparison of results obtained by using the heating and the freezing methods of killing cotton and creosote (*Larrea*) tissues for cryoscopic determinations of the sap concentration shows that the heating process may increase the concentration as much as one atmosphere in some cases. For an extensive series of determinations over a period of time, however, the heating method gives just as reliable an indication of the changes in sap concentration, of certain plant species at least, as does the more expensive and time-consuming method of freezing the tissues.

2. Not only is the heating method reliable for comparative studies of most species, but it has the added advantage of rendering the plant material capable of storage for considerable periods of time without significant change in its sap concentration. Heated samples have been stored for as long as 176 days at room temperature with an average increase in Ov of only 1.4 atmospheres in samples which exhibited differences of as much as 0.8 atmospheres among themselves.

3. The heating method is readily adaptable to extended collections of samples in the field. The samples may be heated in boiling water over a camp stove, or even a camp fire, immediately after collection, and the osmotic values determined after return to the laboratory, without fear of much, if any, change.

4. With the plant material used in these experiments the duration of freezing apparently made little difference, samples frozen 30 minutes giving approximately the same range of values as those frozen for longer periods. MEYER (2) states that a period of at least 8 hours is desirable, and most workers have frozen their material much longer than the 30 minutes required for killing material by heating. This emphasizes the saving of time usually possible by employing the heating technique.

5. The small amount of fuel necessary for killing a group of samples is considerably less expensive than the materials needed for freezing plant material, especially if solid carbon dioxide is used, as is recommended by some workers.

DESERT LABORATORY
TUCSON, ARIZONA

LITERATURE CITED

1. MALLERY, T. D. Changes in the osmotic value of the expressed sap of leaves and small twigs of *Larrea tridentata* as influenced by conditions of the environment. Ecological Monographs (In press).
2. MEYER, B. S. Some critical comments on the methods employed in the expression of leaf saps. Plant Physiol. **4**: 103–112. 1929.
3. WALTER, H. Über die Pressaftgewinnung für kryoskopische Messungen des osmotischen Wertes bei Pflanzen. Ber. deutsch. bot. Ges. **46**: 539–549. 1928.

INFLUENCE OF THYROXIN ON THE GROWTH OF PLANTS

ELIZABETH ELLEN DAVIS

(WITH ONE FIGURE)

Introduction

The general action of thyroxin is probably as a catalyst in the process of combustion, increasing the rate of metabolism. Some work has been done on the effect of thyroxin upon the growth of plants. ABDERHALDEN (1) found that alcoholic fermentation is usually accelerated by either synthetic thyroxin or that of natural origin. He noted, however, that at times the fermentation was retarded. These differences he believed due to the variable condition of the yeast cells used. NIETHAMMER (4) states: "thyroid extract and zinc sulphate, which are known stimulants for seeds, also activate resting buds and to a slight degree cell division." BOSE (2) claims that he obtained a maximum activity in carbon assimilation with thyroid extract at a dilution of one part in a billion. REBELLO (5) in his work on white bulbs of hyacinth found an acceleration of growth when he used dry thyroid powder added to water and when he used 0.5 cc. of 1:500 solution of nucleoprotein and thyroglobulin. When he used several decigrams of fresh thyroid gland in water the bulbs developed as well as the controls which were grown in water. BUDDINGTON (3), in his experiments on bulbs of *Allium* grown in Pfeffer's solution, discovered that the growth of the root tips was retarded in proportion to the amount of thyroid material used, and that potassium iodide in the same amounts as that in thyroid substance had no effect on the root tips; further, he found that the early leaves were not affected by the presence of thyroxin.

Methods and materials

No workers, so far as the writer has been able to determine, have tried the injection method of treating plants with thyroid material; all have used the thyroid material in nutrient solution or in pure water. The method of injection was chosen so that the thyroxin could not react with anything in the nutrient solution or in the soil. For injection it was necessary to choose plants which have hollow stems or which grow from bulbs. *Vicia faba,* the Windsor bean, fulfils the first requirement, *Allium* and *Narcissus* the second. *Pisum sativum* also was used in these experiments.

A cubic centimeter syringe with a fine hypodermic needle was used to make the injections. For the first part of the experiments on *Allium* and a part of those on *Vicia faba,* desiccated thyroid gland was employed, a 2-grain tablet in 20 cc. of water; but in order to escape the possible effects

of the organic material, the experiments were continued with the sodium salt of thyroxin in concentration of 0.4 mg. to 5 cc. water. This material, obtained from Squibb and Sons, is stated to be three hundred times as concentrated as is the desiccated thyroid gland.

The controls were injected with water, and received exactly the same treatment as the experimental plants. Measurements were taken in centimeters and weight in grams. The bulbs of *Allium* were grown in the greenhouse under uniform conditions of temperature, moisture, and light. These and the *Vicia faba* seeds were planted in sandy loam, and the peas and *Narcissus* were grown in nutrient solution or in water. The procedure in the case of the peas differed from that already described in that the thyroxin was added to the water rather than injected into the plants.

Experimental results

Growth of *Allium* from thyroxin-treated bulbs

Medium sized, solid, unsprouted bulbs were chosen and placed in water so that roots might be well started before the injection was made. The bulbs were then injected with thyroid solution and planted in sandy loam. Preliminary experiments were performed using desiccated thyroid gland in concentration of 2 grains in 20 cc. water. The flower stalks developed first on the treated bulbs. In another experiment using 12 bulbs, 5 cc. of the solution of desiccated thyroid gland were injected. The plants from the treated bulbs were depressed in vegetative growth, varying on different observations from 7.7 to 28 per cent. The height of the tallest leaves was taken as a measure of growth. Flowering occurred sooner in the treated bulbs, with the exception of two sets of treated and controls which did not flower at all. In one set the flowering took place three weeks earlier in the treated bulbs; and in three sets one week earlier.

In the second group of *Allium* the method was the same but more plants were used, and the weights of the roots were recorded so that more conclusive results might be obtained. A solution of the strength 0.4 mg. sodium salt of thyroxin in 5 cc. water was injected into 36 bulbs and water into 36 bulbs. The doses varied from 1 to 5 cc., but there was no real difference in the development of the three series, hence the figures for all are here combined. Considering height as a measure of growth, table I and figure 1 indicate that the treated material showed a decrease until the sixtieth day after planting, at which time they began to gain in height over the controls. On the sixtieth day after planting, all of the leaves of each plant were measured. The treated averaged one leaf less per plant than the controls but the heights of the plants were approximately the same.

The number of flower stalks was slightly greater among the treated plants: six more than the controls at 88 days after planting, and five more

TABLE I

HEIGHTS OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN

NUMBER OF DAYS AFTER PLANTING	TREATED		CONTROL		PERCENTAGE DIFFERENCE IN TREATED PLANTS
	NUMBER OF PLANTS	AVERAGE HEIGHT	NUMBER OF PLANTS	AVERAGE HEIGHT	
		cm.		*cm.*	%
32	35	9.93	35	12.06	−17.06*
46	34	26.79	36	37.27	− 1.76
60	34	39.19	36	38.16	+ 2.72
74	34	47.50	33	43.15	+ 10.10
88	34	49.92	33	46.42	+ 7.50

* Plus sign indicates that the treated showed greater growth than the controls, the minus sign indicates that they exhibited less growth.

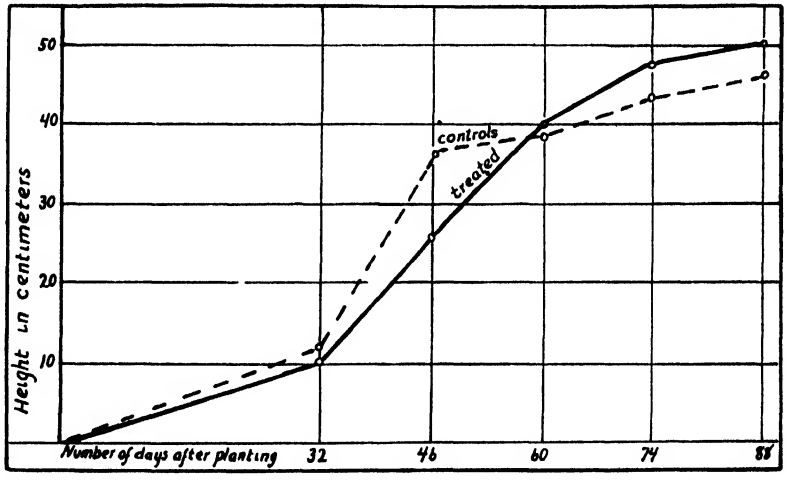


FIG. 1. Heights of *Allium* plants from thyroxin-treated bulbs. The treated plants showed a decrease for the first 60 days but after that they surpassed the controls slightly. The controls had an average of one more leaf per plant.

at 102 days after planting. At the final measurement, 102 days after planting, the treated plants averaged about 34 cm. in height and the controls about 21 cm. The opening of inflorescences took place sooner and progressed more rapidly in the treated than in the controls, as shown in table II. One hundred and sixteen days after planting, the treated showed almost three times as many open inflorescences as the controls.

The roots were cut from the bulbs, washed, and weighed. Table III shows that the average green weight of roots per plant of the treated was 25.87 per cent. less than that of the controls. The weight of the air-dried

TABLE II

OPENING OF INFLORESCENCES OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN; 34 EACH OF TREATED PLANTS AND CONTROLS

NUMBER OF DAYS AFTER PLANTING	NUMBER OF INFLORESCENCES OPEN	
	TREATED, 34 PLANTS	CONTROLS, 34 PLANTS
96	2	0
100	6	1
105	8	3
110	9	3
116	17	6
123	20	12
130	20	16
137	21	17

TABLE III

WEIGHT OF ROOTS OF *ALLIUM* PLANTS FROM BULBS TREATED WITH THYROXIN

NUMBER		AVERAGE GREEN WEIGHT		DEPRESSION OF TREATED	AVERAGE DRY WEIGHT		DEPRESSION OF TREATED
TREATED	CONTROL	TREATED	CONTROL		TREATED	CONTROL	
		gm.	*gm.*	%	*gm.*	*gm.*	%
31	32	16.67	22.49	25.87	1.45	1.83	20.76

roots was consistent with the green weight; the treated showed a depression in weight of 20.76 per cent.

These experiments as a whole show that thyroxin does not have any consistent effect on green tops of *Allium*, but has a well marked accelerating influence on the time of flowering and a stunting effect on the roots.

GROWTH OF *NARCISSUS* AND *VICIA FABA* AS INFLUENCED BY THYROXIN

The experiment on *Narcissus* was conducted in the same manner as those on *Allium*, except that the bulbs were grown in water instead of in soil. Seventeen bulbs were injected with 2.5 cc. of solution made by adding 0.4 mg. sodium salt of thyroxin to 5 cc. water. The treated individuals showed a somewhat better vegetative growth and the inflorescence opened sooner. All of the inflorescences of the treated material were open three days before all of the inflorescences of the controls. These results correspond with those found in the case of *Allium*, except for the fact that some of the plants from treated bulbs of *Allium* showed a depression in vegetative growth whereas others showed acceleration.

Vicia faba material was treated by injecting thyroxin solution into the

youngest internode, or the next youngest in case the epidermis broke. Injections were made each week for seven weeks with fresh solutions. It was impossible to determine just how much of the solution remained within the stem, but the minimum was not less than 0.1 cc. In all, 42 plants were studied, 20 grown in the greenhouse and 22 out-of-doors. The results in both cases were approximately the same, although the plants grown out-of-doors were, as would be expected, consistently shorter than those grown in the greenhouse. The treated showed a depression in growth up to 9.99 per cent. Flowering and formation of pods occurred at approximately the same time in the treated and the controls.

Growth of *Pisum sativum* in solutions containing thyroxin

Experiments were carried out to determine whether or not thyroxin has a stunting effect on the growth of peas. Buddington (3) found that the roots of onions grown in Pfeffer's solution with thyroxin added were less well developed than those grown in Pfeffer's solution with thyroxin lacking. Blue Bantam peas were soaked in water for 24 hours and then placed in glass germinators. When the roots were well started the seedlings were placed in 500-cc. jars filled in the first experiment with Pfeffer's solution and in the second with water. In each case 1 mg. sodium salt of thyroxin

TABLE IV

Pisum sativum grown in water with thyroxin added

	Treated, 50 plants	Control, 50 plants	Percentage difference in treated plants
Green tops			%
Total height (cm.)			
After 12 days	327.60	316.60	+ 3.47*
After 26 days	624.00	619.50	+ 0.72
Average height (cm.)			
After 12 days	6.53	6.33	+ 3.25
After 26 days	12.48	12.39	+ 0.72
Total green weight (gm.)	41.00	36.25	+13.10
Average green weight (gm.)	0.82	0.73	+13.10
Roots			
Total length (cm.)	563.00	762.00	−12.99
Average length (cm.)	11.26	15.24	−26.11
Total green weight (gm.)	28.40	27.00	+ 5.18
Average green weight (gm.)	0.57	0.54	+ 5.18

* Plus sign indicates that the treated showed greater growth than the controls, the minus sign that they exhibited less growth.

was added to half of the jars. The results of the experiment in which Pfeffer's solution was used are not fully recorded here, for a slimy bacterial film collected about the roots. In general the controls showed a somewhat greater growth. The results of the experiment in which water was used are recorded in table IV. Here the treated show the greater growth and greater green weight. The length of the primary root of the treated was shorter, as shown in table IV. The roots of the treated were slightly thicker, less branched, and heavier than the controls in spite of the stunting in length. However, the presence of a film on the roots of the treated material may have added slightly to the weight, although the roots were thoroughly washed and dried.

The experiments with peas showed as a whole that thyroxin had no noticeable influence on the length of the tops, but did cause a shortening of the roots. The treated plants exhibited increased weight but probably not enough to be significant.

Discussion

The investigation of the influence of ductless-gland secretions on the growth of plants has not drawn many workers. Those who have experimented in this field have grown plants in a nutrient solution or in water to which the thyroid material was added. Such a plan can hardly be satisfactory, since it is possible that the thyroid material may react with substances in solution, or that bacterial action in a solution containing thyroxin may influence growth. The present study for the most part made use of the injection method in order to avoid these possible difficulties.

The time of flowering of *Allium* and *Narcissus* was accelerated by the presence of thyroxin. At one time in the development of *Allium* there were three times as many open inflorescences among the treated plants as among the controls. Treated *Allium* plants had about 23 per cent. more inflorescences open, and 16 per cent. more flower stalks 102 days after planting. The flower stalks of the treated bulbs of *Allium* averaged at least 50 per cent. taller. There was not much difference in the time of opening of the inflorescences of the treated and untreated bulbs of *Narcissus,* but there was a three-day interval from the opening of the first inflorescence of the treated material before any controls blossomed. This acceleration is to some degree substantiated by the results of REBELLO (5), who found that the white bulbs of hyacinth were accelerated in growth; and in principle is affirmed by the results of NIETHAMMER (4), who found that resting buds were activated by thyroxin.

The roots of *Allium* and *Pisum* were stunted in the thyroxin solutions. The method of treating the plants was different in the two cases but the results were much the same. The weight of the roots of the treated bulbs

of *Allium* was at least 20 per cent. less than that of the controls. The extra weight may have been due partly to the film which developed on the roots. The stunting effect agrees with the results obtained by BUDDINGTON, who found that the root tips of *Allium* were retarded in growth by thyroxin.

The majority of observations indicate that thyroxin has a somewhat depressing effect on green vegetative growth of *Allium, Narcissus,* and *Pisum sativum* but the results were variable at different dates and with different plants. *Narcissus* showed an acceleration in growth of the treated at every date of observation; *Vicia faba* showed a depression in 75 per cent. of the observations; *Pisum* grown in Pffeffer's solution containing thyroxin was depressed in 88 per cent. of the cases.

Summary

1. *Allium* and *Narcissus* bulbs and *Vicia faba* plants were treated with injections of thyroid material, while *Pisum sativum* seedlings were grown in solutions containing thyroxin.

2. The time of flowering of *Allium* and *Narcissus* was hastened in the thyroxin-treated plants. *Allium* plants showed a stimulation of at least 16 per cent. in time of flowering and in height and number of flower stalks. Acceleration in the flowering time of *Narcissus* occurred also but was not so marked.

3. Roots of treated *Allium* plants were lighter in weight by 20 per cent. than the controls. Roots of the *Pisum* material were shorter by 26 per cent. than those of the controls but showed greater weight, possibly because of adhering bacterial film.

4. Green vegetative parts in general were depressed in growth among the treated plants, although they sometimes showed an early temporary acceleration, especially *Narcissus* and *Pisum.*

These experiments were carried out in the greenhouse of the University of Colorado under the supervision of Professor EDNA L. JOHNSON; to her and to Professor FRANCIS RAMALEY the writer is indebted for valuable suggestions in the experimental work and in the preparation of the manuscript.

UNIVERSITY OF COLORADO
BOULDER, COLORADO

LITERATURE CITED

1. ABDERHALDEN, E. Influence of synthetic thyroxin and of that prepared from the thyroid gland on alcoholic fermentation. Fermentforschung **9**: 243–245. 1927.

2. BOSE, J. C. Effect of infinitesimal traces of chemical substances on photosynthesis. Nature **112**: 95–96. 1923.
3. BUDDINGTON, R. A. The influence of certain ductless gland substances on the growth of plant tissues. Biol. Bull. **37**: 188–193. 1919.
4. NIETHAMMER, ANNELIESE. Die Beeinflussung ruhender Knospen und der Zellteilung durch Thyreoidea und Zinksulfat. Protoplasma **2**: 392–400. 1927.
5. REBELLO, SILVIO. Action des glandes a sécrétion interne et de leurs extraits sur le développement des plantes. Compt. Rend. Soc. Biol. **90**: 1095–1097. 1924.

BRIEF PAPERS

EFFECT OF HIGH FREQUENCY SOUND WAVES ON OXIDASE ACTIVITY

(WITH ONE FIGURE)

During the last few years numerous papers have appeared dealing with the chemical and biological effects of high frequency sound radiation. In the present investigation its effects on the activity of oxidase have been determined.

The apparatus producing high frequency sound radiation was similar to that used by HARVEY, HARVEY, and LOOMIS (**2**). The waves were produced by a quartz crystal measuring 2.5 cm. square and ground to give a natural frequency of 450,000 cycles. It was immersed in an oil bath, supplied with an adequate cooling system. This crystal was energized by means of a step-up air transformer, the primary of which was the tank coil of an oscillating Hartley circuit. The plate of the 75-watt tube was supplied with a 60-cycle unrectified current at a voltage of 3500 (r.m.s.).

Fruit extracts were prepared from apricots, peaches, and avocados. The fruit was frozen, ground, dispersed in a large volume of water, and filtered. The oxidase activity of these extracts as measured by the rate of oxygen absorption in the presence of catechol was similar to that of oxidase preparations obtained by the alcohol precipitation method.

Five cc. of the extract were introduced into a thin-walled bulb, which was suspended in the oil bath so that its bottom was about 5 mm. above the crystal. Samples were withdrawn from time to time and their oxidase activity determined by means of a WARBURG respiration apparatus (**3**). The rate of oxygen absorption at 25° C. was measured, using an acetate buffer of pH 4.9 in the presence of 1.5 per cent. of catechol as substrate.

In all cases exposure to the sound waves resulted in a considerable decrease of oxidase activity. No complete inactivation was obtained in treatments up to 12 hours. When the time of exposure was plotted against oxidase activity, all curves approached a logarithmic form; however, the data were not strictly reproducible with the equipment used. A typical curve is shown in figure 1.

In order to correct for effect of temperature, controls were incubated at 38° C., the highest temperature observed in the exposed tube. As shown in the figure, the decrease in activity at this temperature was negligible in comparison with that produced by high frequency sound waves.

The action of sound waves has been frequently ascribed to oxidative processes. It was found, however, that washing out most of the air with

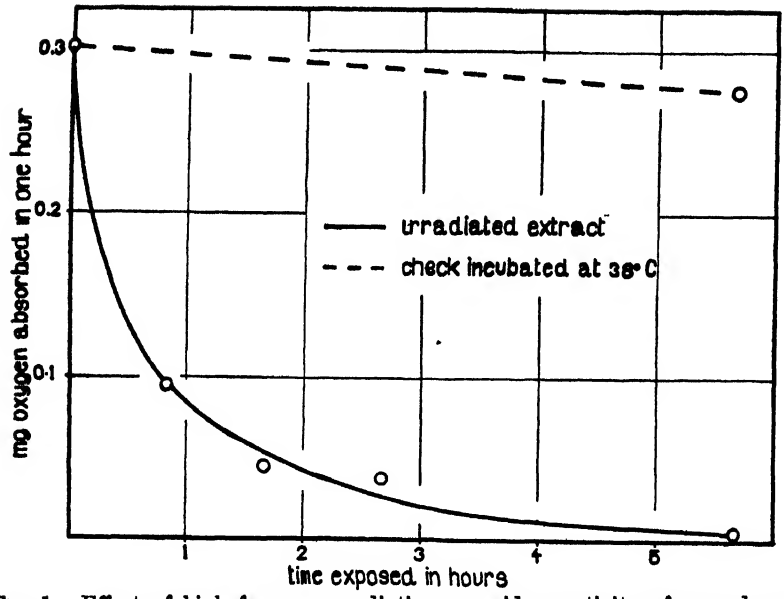


FIG. 1. Effect of high frequency radiation on oxidase activity of avocado extract under nitrogen.

hydrogen or nitrogen by bubbling the gas through the solution before treatment, and also by continuing the washing during the treatment, produced no significant differences in the effect of irradiation.

Since recently BEUTHE (1) has attributed the effect of high frequency sound waves to the formation of hydrogen peroxide, the amount of H_2O_2 formed during irradiation of distilled water was measured by iodine titration. When 100 times this concentration of H_2O_2 was added to the fruit extract, its oxidase activity was not affected appreciably.

It is seen that high frequency sound waves progressively destroy oxidase activity. It would appear that oxidation played a minor rôle in this destruction.—RALPH J. CHRISTENSEN and RUDOLF SAMISCH, *Fruit Products Laboratory, University of California.*

LITERATURE CITED

1. BEUTHE, H. Über den Einfluss der Ultraschallwellen auf chemische. Prozesse. Zeitschr. phys. Chem. Abt. A. **163**: 161–171. 1933.
2. HARVEY, E. N., HARVEY, E. B., and LOOMIS, A. L. Further observations on the effect of high frequency sound waves on living matter. Biol. Bull. **55**: 459–469. 1928.
3. WARBURG, O. Manometrische Messung des Zellstoffwechsels in Serum. Biochem. Zeitschr. **164**: 481–503. 1925.

LATERAL WATER TRANSFER IN LEAVES OF *GINKGO BILOBA*

(WITH ONE FIGURE)

Some years ago, while the writer was investigating the mass factor[1] in the energy relations of leaves, an interesting observation was made on the lateral transfer of water in the leaf tissues of *Ginkgo biloba*. When the leaves of *Ginkgo*, still attached to the twig, had been perforated with the Ganong leaf punch, it was noticed that the tissues distal to the cut died from desiccation within 24 to 48 hours. The edges of the dead tissue ran parallel to the venation of the leaf, but a certain amount of lateral transfer of water from uncut veins into the region whose water supply had been cut off was observed. At that time it was not possible to follow up this interesting problem.

Punching the leaves of ordinary dicotyledonous net-veined leaves does not greatly hamper water distribution throughout the leaf. Leaflets of the bush honeysuckle (*Lonicera morrowi*), for instance, have been cut transversely near the base of the leaflet in such a manner as to sever the midvein water supply, and to leave less than a millimeter of uncut tissue on each flank of the leaflet. Such cut leaflets, if supported mechanically so that they are not torn by air movement, remain green and turgid for days, showing that water enough for the entire leaf can be distributed to the tissues beyond the cut, through the very small uncut basal marginal vein system. It should be extremely interesting to observe under a microscope the distribution of dyes in leaves thus injured.

Ginkgo, with its dichotomous, parallel venation, and with no connection between the veins except mesophyll cells, presents an entirely different problem when the veins are severed transversely. In such a case the transfer of water into the tissues distal to the severed region must be accomplished by a lateral diffusion of water from the nearest uncut veins. To determine how far water can be transmitted laterally through the mesophyll cells with speed sufficient to maintain life, leaves were cut during the seasons 1931, 1932, and 1933, and some measurements made.

In figure 1 are shown some of the leaves after they had developed the necrotic areas after transverse cutting of the veins. In leaf no. 1 the cut is about 10 mm. long; in no. 2, 5 mm.; no. 3, 4 mm.; and no. 4, 2.5 mm. Leaves cut to a less degree than no. 4 did not show death of tissues beyond the severed region. As may be clearly observed in the photograph, the water travels laterally and maintains the life of the tissues to a somewhat variable distance. Careful measurements from the first uncut vein on

[1] SHULL, CHARLES A. The mass factor in the energy relations of leaves. Plant Physiol. 5: 279–282. 1930.

either side to the edge of the tissue which is killed show that the lateral diffusion of water is rapid enough to supply the cells in certain cases to a distance of 2.6 to 3 mm. In traversing this distance the water passes through three or four interveinal tracts, as the vascular bundles are usually less than 1 mm apart In leaf no. 4 the dead tissue at the narrowest point seems to represent just one interveinal tract. In this instance the lateral transfer is effective through a shorter distance than in leaf no. 1. The leaves vary considerably in their ability to transmit the water through the mesophyll cells

The results seem to depend somewhat on the age and condition of the leaf at the time of cutting, and its position on the tree. In more exposed situations the tissues die to a greater extent than in less exposed situations Young leaves seem to transfer water laterally farther than old leaves. In some cases young leaves seemed to become adjusted, and transmitted the water farther than would have been the case if the leaves had been cut after a greater degree of maturity had been attained. Possibly the cells retain thinner walls, and maintain a more permeable protoplasm in this region, when the cut is made early. To establish this point would require a careful investigation of the aging of leaf tissues; and a comparative study of protoplasmic permeability in young and old leaves, and in old regions which had been isolated by cutting while young. No such studies have been made.

The distance to which water could be transported laterally and successfully maintain cell turgor was much less than had been anticipated. The experiment is one that lends itself very readily to elementary class instruction, and requires only a *Ginkgo* tree, a pen knife, and a millimeter rule. The contrasting behavior of net-veined leaves is easily established by similar methods.—CHARLES A. SHULL, *University of Chicago.*

COMPARISON OF ANATOMICAL AND HISTOLOGICAL DIFFERENCES BETWEEN ROOTS OF BARLEY GROWN IN AERATED AND IN NON-AERATED CULTURE SOLUTIONS

It has been observed by workers on absorption problems in this laboratory that the roots of barley grown in an aerated culture solution have a strikingly different growth habit from those grown under non-aerated conditions. The primary roots of the former are several times as long and the secondaries less numerous. This paper is a preliminary report of a study undertaken to ascertain whether there were any well defined anatomical or histological differences accompanying these different types of root systems.

Two culture tanks made of black sheet iron coated inside with a non-toxic asphaltum paint were used, each tank having a capacity of 112.4 liters. Hoagland's culture solution supplied the necessary inorganic salts, the original volume in each tank being maintained by the addition of distilled water to replace the loss due to transpiration. In one tank aeration was effected by means of fine continuous streams of washed air from an air compressor; in the other the oxygen supply was limited to that diffusing downward from the surface of the culture solution.

A pure strain of barley of the Sacramento variety was employed, a great number of seeds being germinated in the usual manner, and from these the necessary number of seedlings selected for the experiment. Selection was made on the basis of uniformity of size, number of leaves, length of leaves, etc. Thirty-two such plants were grown in each tank.

Once every week for a period of two months two plants were taken from each culture tank. The leaves were counted and the length of each leaf measured. The whole root system was cut off and floated in a large tank of water. With care the roots could be separated, counted, and their lengths measured. The number of roots refers to those emerging from the stem plate or crown. At each sampling transverse sections for microscopic examination were prepared from at least ten different roots taken from the plants grown in the aerated culture solution, and a like number from those grown in the non-aerated solution. The sections were taken from the following positions back from the root tip: 5, 15, 25, 35, 45, 55 mm., and at

the top of the roots. They were stained and made up into permanent slides. A careful study was made of the prepared slides and the following method of examination was adopted. The diameter of the roots was measured, as was also the thickness of the cell walls of the cortex, endodermis, pericycle, xylem vessels, and the large central vessels. The measurements were all made with oil immersion.

The results of the macroscopic examination may be summarized as follows:

1. During the first month all of the plants had about the same number of roots. After this period the number of roots produced by the non-aerated plants increased very rapidly, whereas those of the aerated increased but slightly, and at the end of sixty days the plants growing in the non-aerated culture solution had an average of 225 roots and those grown in the aerated culture solution an average of 75 roots.

2. Differences in root length became evident within a few days after planting, and at the end of sixty days the average length of the roots in the aerated solution was 37.4 cm. as against 10.9 cm. for the non-aerated plants.

3. The roots of the non-aerated plants were about 15 per cent. greater in diameter throughout their entire length than were those of the aerated plants.

4. In general the tops seem to show the same tendencies which were so evident in the root system, *i.e.*, the shoots of the non-aerated plants consisted of a somewhat greater number of shorter leaves than did those of the aerated plants. But the shoot is not influenced by root aeration to the same extent as is the root itself; in fact, individual differences between the tops of plants grown in the same culture solution may be as great as those of plants grown in the different culture solutions.

No weights were taken of the plants described in this paper, but other workers in this laboratory have shown that there is no significant difference in the weights of either the tops or roots of barley plants grown in aerated and non-aerated culture solutions when aeration is effected as described here.

The results of the microscopic examination may be briefly summarized as follows:

1. The cortex of the roots grown in the aerated culture solution consists of uniformly compact parenchyma with no conspicuous intercellular spaces. The cortical region of the roots grown in the non-aerated solution is composed of large air passages separated by narrow strands of parenchyma.

2. The first tissue to differentiate is the xylem vessels of the non-aerated roots. These vessels develop secondary thickening of their walls at a distance of 5 mm. from the root tips. The next is the xylem vessels of the aerated roots at a distance of 15 mm. from the root tips. This is followed

by the pericycle of the non-aerated roots at a distance of 35 mm. from the root tips. At a distance of 45 mm. from the root tips the cell walls of the pericycle of the aerated roots have begun to thicken, as have also those of the central ducts of the non-aerated roots. At a distance of 55 mm. from the tips the cell walls of the endodermis of both the aerated and non-aerated roots have begun to thicken. At the top of the roots next to the crown all of the walls included in the measurements have become secondarily thickened, and the thickness of the cell walls of the roots grown in the aerated culture solution is about twice as great as that of those grown in the non-aerated solution.

It has been shown by others in this laboratory that the concentration of reducing and total sugars of barley roots grown in an aerated culture solution is less than that of those grown in a non-aerated solution. It seems reasonable to assume that the greater amount of oxygen available to the aerated roots, with its consequent accelerating effect on the respiratory rate, decreases the sugar concentration to a point where it becomes the limiting factor in cell wall thickening, particularly so in the lower root fraction. The cell walls of the root near the crown are not so limited, since sugar depletion has not yet taken place to any great extent. Here the greater thickening may reflect a higher level of protoplasmic activity due to a greater oxygen as well as to an adequate sugar supply.

The greater length of the aerated roots may also be accounted for by the greater supply of oxygen available to their apical meristems.

Very briefly, then, the tissues in the roots grown in the non-aerated culture solution start to differentiate nearer the root tip than do those grown in the aerated culture solution. But, starting at about 25 mm. from the root tips, the cell walls of the roots grown in the aerated culture solution start to thicken more rapidly than do those of the plants grown in the non-aerated culture solution, and in the mature regions of the roots these walls are about twice as thick as the corresponding cell walls of the roots grown in the non-aerated solution. These structural and sugar content differences probably have their explanation in the differences in amounts of oxygen in the two culture solutions.

The writer wishes to acknowledge his indebtedness to Professor A. R. DAVIS for the valuable advice received during the course of this work.—A. E. BRYANT, *Laboratories of Plant Nutrition and the Department of Botany, University of California.*

TOLERANCE OF LIQUID-AIR TEMPERATURES BY SEEDS OF HIGHER PLANTS FOR SIXTY DAYS

In 1897 BROWN and ESCOMBE[1] published results of experiments which proved that seeds of a number of different higher plants were uninjured by exposure to liquid-air temperature (−189° to −193° C.) for 110 hours. Later THISELTON-DYER[2] and BECQUEREL[3] published confirmatory evidence to the same effect and in addition THISELTON-DYER demonstrated that several different kinds of seeds tolerated, without visible injury, the temperature of liquid hydrogen (−250° C.) for one to six hours. It occurred to us that it would be interesting to learn whether or not seeds can tolerate liquid-air temperatures for much longer periods than 110 hours. Accordingly, we selected nineteen varieties of seeds of higher plants, dried them for one week over calcium chloride, and distributed them into three glass tubes which were immersed in liquid air. One tube was removed from the liquid air after thirty days and the other two tubes after sixty days. After removal from the liquid air the tubes were opened and the seeds tested for germinating capacity alongside of control seeds from the same original lot which had not been subjected to liquid-air temperature. Within twenty-four hours after the seeds were placed between layers of wet filter paper many were germinating, and equally well in the treated and in the control specimens. After a few days practically all seeds of most of the species represented had germinated into vigorous seedlings. Two or three species showed poor germinating capacity, but this was true alike for the control and the treated seeds of those varieties. In order to determine whether or not injury was sustained by seeds at liquid-air temperature which was not apparent during the germination period, seedlings from the seeds tolerating the sixty-day exposure to liquid air are being grown in culture solutions alongside of control seedlings of the same varieties. These have now (30th January, 1934) been growing for about ten weeks in a greenhouse, and there is not the slightest evidence of injury by the liquid air treatment for so long a period as sixty days. In some instances the seedlings from the treated lot of seed are somewhat superior to the controls in amount of growth attained and in other cases the reverse is true, but no significant differences are discernible.

The kinds of seeds treated for thirty days are as follows:

[1] BROWN, H. T., and ESCOMBE, F. Note on the influence of very low temperatures on the germinative power of seeds. Proc. Roy. Soc. London 62: 160–165. 1897.

[2] THISELTON-DYER, SIR W. On the influence of liquid hydrogen on the germinative power of seeds. Proc. Roy. Soc. London 65: 361–368. 1899.

[3] Becquerel, Paul. Recherches sur la vie latente des graines. Ann. Sci. Nat. Series 9, 5: 222–228. 1907.

Early amber sugar-cane	Kenota oats
Stenning spinach	Silver-skinned white Portugal onions
Early white spine cucumbers	Milo maize
Sugar beets	*Melilotus indica*
Japanese buckwheat	Yellow mustard
Sacramento barley	Santa Clara tomatoes
Purple vetch	

In the sixty-day series all of the foregoing seeds were tested and in addition the following:

Canadian peas	Hubbard squash
Golden bantam corn	Grimm alfalfa
American Wonder peas	Russian mammoth sunflower

Many interesting questions arise in one's mind on being confronted by such results as those above described. While space in this paper will not permit a discussion of them all and since we intend to discuss these problems later, mention may be made of only two or three aspects of such an inquiry. Inasmuch as seeds may be immersed in liquid air for sixty days without injury, as we have shown, one might ask the question as to whether or not it would be possible for the life in seeds to be preserved forever at liquid-air temperatures. On the basis of what we know today regarding the longevity of seeds under other conditions than liquid-air temperatures, we are inclined to answer this question in the negative, because no studies have yet been made of any seed, with the exception of *Nelumbium,* which has been shown to have life in it for as great a period as a century. In the experiments with *Nelumbium* it will be recalled that a seed at the British Museum 150 years old was made to germinate and that OHGA found *Nelumbium* seeds which he believed to be several centuries old, all of which were found to have germinating capacity. This leaves the question open as to whether or not some seeds, even though it may not be true of all, may have power to resist deterioration indefinitely. It must also be said, however, that there is another side to this problem, namely, that since most seeds which we know today have a relatively short life, the destruction of enzymes and similar substances or agencies in the seed may lead to the abbreviation of life in seeds quite apart from the question of whether or not the embryo retains its respiratory powers. If, however, it should prove to be the case that at liquid-air temperatures respiration is either extremely slow or nonexistent, and further that the enzymes and other substances to which I have referred, or other processes concerned with the living seed, remain unchanged within the seed, then keeping seeds at such temperatures would indeed be a way of preserving them forever. Further research may perhaps yield some results which will lead to a more definite answer to this problem.

Another aspect of the subject from which our results emanate naturally comes to mind now, namely, the condition of the water in the seeds which are exposed to liquid-air temperatures. Obviously it is water which does not freeze, for if it did freeze it is in the highest degree likely that the seeds would be injured. In other words, therefore, such seeds as we have used with approximately ten per cent of water in them contain the water in the form commonly spoken of as "bound water." To be sure, the term "bound water" is differently defined by different investigators. Nevertheless, we may regard bound water in this case as water which is contained in spaces that are so minute as to render impossible the behavior of that water in the way in which water in large spaces does behave at low temperatures. Just as it is in the highest degree likely that water will not freeze in extremely fine capillary tubes, so water contained in the very minute spaces or interstices of the colloids making up the embryo and the balance of the seed would also be proof against freezing at any temperature, no matter how low.

We are indebted to Dr. G. R. MACDONALD for keeping the supply of liquid air replenished during the experiment and to Mr. W. C. CHANDLER for assistance in the germination tests and in the subsequent culture experiments.—CHAS. B. LIPMAN and G. N. LEWIS, *University of California.*

NOTES

Summer Meeting.—A summer meeting of the Society in cooperation with other botanical groups will be held at Berkeley, California, in connection with the summer meeting of the A. A. A. S. Professor J. P. BENNETT, of the University of California, has been appointed to take charge of all local arrangements in connection with this meeting. It affords opportunity for the western members to get together, and a large attendance is expected. The general headquarters will be at the Stephens Union Building. There will be appropriate symposia and joint meetings with the Botanical Society of America and Section G of the A. A. A. S. The outlook is for an unusually good meeting, and abundant opportunity will be provided for visits to the Bay cities, Mount Tamalpais, and the surrounding country. Stanford University and Davis are not too far away for a visit during the period of the meetings, June 18–23, 1934.

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists will be held at Pittsburgh in December, 1934. The local representative is Dr. O. E. JENNINGS, of the University of Pittsburgh. It is not too early to begin planning for the meeting. Situated between east and west, it should not be difficult to set attendance records, and to find a wealth of material from which to select for the programs.

Minnesota Section.—The Minnesota Section has several meetings planned for the spring months. The titles of the papers to be presented and the speakers are as follows:

April 4, 1934, Physiology and anatomy of the embryo sac. F. E. BUTTERS.

May 9, Mineral nutrition. FREDERICK CHANDLER.

June 6, Physiological effects of alcohol. CHARLES ROGERS.

The meetings are always open to those who are not members of the Society, and interested non-members are cordially invited to attend the meetings.

New England Section.—The New England Section is starting its work by sponsoring a meeting at Amherst, Massachusetts, on May 25–26, 1934, from Friday noon to Saturday noon. It is the desire of the local committee that all plant physiologists in this region should attend, and an invitation is extended to all persons interested in botany, plant pathology, horticulture, forestry, agronomy, etc., to meet with them. Anyone is privileged to present a paper. It is hoped that through this sponsorship the plant physiologists and their friends may join in a period of good fellowship, and in

the enjoyment of the interesting programs being arranged for this meeting. Titles should be sent to LINUS H. JONES, secretary of the section, Clark Hall, Massachusetts State College, Amherst, Mass.

Purdue Section.—The Purdue Section reports an interesting series of meetings for 1933–1934. Twelve meetings were arranged for the year with the following programs:

October 16, 1933, The caustic and stimulative effects of x-rays on plants. C. A. SHULL, guest speaker.

November 3, MITSCHERLICH's quantitative method of using plants to determine plant nutrient deficiencies of soils. S. R. MILES.

November 20, The biology of mixed cultures of microorganisms. C. L. PORTER.

December 4, Some effects of soft x-rays on seedlings. H. M. BENEDICT.

January 15, 1934, Reports on the Boston meetings, A. A. A. S.

February 5, Movies taken in Honduras. H. E. ENDERS.

February 19, The chemistry and physiological importance of carotene and related pigments. C. L. SHREWSBURY.

March 5, Root growth habits in relation to performance of inbred and hybrid corn. R. R. ST. JOHN.

March 19, Fruit setting as a physiological problem. LAURENZ GREENE.

April 2, Effect of supplementary artificial radiation on plant growth. R. B. WITHROW.

April 19, Effect of light on the carbohydrate and nitrogen metabolism of asters. RAYMOND WENGER.

May 7, Guest speaker, unannounced.

The attendance at the meetings has varied from 20 to 40, with an average of about 25. The meetings are informal, with an attractive combination of social and scientific interest. During the year G. N. HOFFER has been president of the section, R. B. WITHROW secretary-treasurer, and RALPH M. CALDWELL chairman of the program committee.

Program Committee.—The program committee has been appointed by the president, Dr. C. O. APPLEMAN of Maryland. The members on the committee are as follows: Dr. H. C. SAMPSON, Ohio State University; Dr. P. D. STRAUSBAUGH, West Virginia University; Dr. WARREN B. MACK, Pennsylvania State College; and Dr. H. R. KRAYBILL, Purdue University, chairman. Dr. A. E. MURNEEK, University of Missouri, is *ex officio* member of the committee. Cooperation of all members of the Society in any matters pertaining to the meeting will be greatly appreciated.

Stephen Hales Prize Committee.—*The fourth award of the* STEPHEN HALES *prize will be made at the Pittsburgh meeting. The committee of* award according to the by-laws is constituted as follows: Dr. D. R. HOAGLAND, University of California, chairman; Dr. W. W. GARNER, U. S. Department of Agriculture; and Dr. H. B. VICKERY, Connecticut Agricultural Experiment Station. These three members, recipients of the award in past years, have the unique privilege of selecting the fourth investigator to be honored by the Society in this manner.

Chemical Methods Committee.—The personnel of the Chemical Methods committee has been modified by the appointment of Dr. Z. I. KERTESZ of the New York Agricultural Experiment Station (Geneva) as a member of the committee. This committee is engaged in preparation of a supplementary report on chemical methods.

Recent Advances in Plant Physiology.—A second edition of E. C. BARTON-WRIGHT'S summary of the recent advances in plant physiology is now available. It is published by P. Blakiston's Son and Co., Philadelphia. There are some changes in organization from the first edition. The discussion of soil is omitted, and the nine chapter headings are: Absorption of water and transpiration; carbon assimilation; nitrogen metabolism; the raw materials of plant nutrition; translocation; respiration; growth; light and growth; and accessory growth factors and related problems. The work covers approximately the last 15 years of progress. The price is $4.00 per copy.

Thermodynamics of Plants.—An interesting and valuable monograph (no. 30 of the Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere), Pflanzenthermodynamik, by KURT STERN, has been published by Julius Springer, Berlin. The first half of the monograph deals with the physical basis of plant thermodynamics; the fundamental laws of thermodynamics; phase rules and phase transformations; and the general nature of chemical, electrical, radiant, and surface energy. The second half of the volume is devoted to applications of these principles to the physiological processes of plants. This is the most useful portion of the book, but the general principles must be thoroughly mastered before the applications can be appreciated. The applicability of the first and second laws; the thermodynamics of phase transformations; and the thermodynamics of the chemical and electrical processes of plant cells are covered. There is a special chapter on thermodynamics of carbon assimilation, and one on surface phenomena. The final chapter is a general summary. Those who are interested will find a wealth of information brought together

in illuminating fashion. The price of the work in brochure binding is RM 32, bound RM 33.2 per copy.

Progress of Botany.—The second volume of the Fortschritte der Botanik which is edited by FRITZ VON WETTSTEIN, München, was published late in 1933 by Julius Springer, Berlin. It covers the main advances for the year 1932. The fields summarized are morphology; systematics, paleobotany, and genetic plant geography; physiology of metabolism; physiology of growth, heredity, and development; and a brief supplement devoted to ecology. There are sixteen collaborators responsible for the seventeen individual sections. This annual is a valuable work for both teacher and investigator. It assists one in maintaining a bird's-eye view of the progress of botany, and its current trends. The quoted price is RM 24 for brochure binding.

Researches on Fungi.—Volume V of A. H. REGINALD BULLER'S work, Researches on Fungi, was published late in 1933 by Longmans, Green and Co. Among other things the author gives an account of the translocation of protoplasm through the septate mycelia of higher fungi and the causes of such translocation. He thinks that the new facts brought to light in this work may have bearing upon the problem of translocation of organic substances through the sieve tubes of the higher plants. Physiologists will find many interesting observations in the volume. The price of Volume V is 26 shillings.

Vol. 9 No. 3

PLANT PHYSIOLOGY

JULY, 1934

SOME EFFECTS OF POTASSIUM UPON THE GROWTH OF SUGAR CANE AND UPON THE ABSORPTION AND MIGRATION OF ASH CONSTITUENTS[1]

CONSTANCE ENDICOTT HARTT

(WITH ONE PLATE AND TWENTY-NINE FIGURES)

Introduction

An account was published in 1929 (**28**) dealing with the effect of varying amounts of potassium upon the growth, enzyme activity, moisture percentage, sugar content, cellular structure, and microchemistry of the sugar cane, variety Louisiana Purple. Suggestions were offered regarding the causes of the symptoms of potassium starvation, and an attempt was made to connect as cause and effect several derangements in the morphology and the physiology of sugar cane which were found to accompany the lack of potassium. The object of the present investigation was to repeat and expand the former study and thus to approach a better understanding of the rôle of potassium in the sugar cane plant.

The research program, of which this is the first report, included determinations of the enzymes invertase, amylase, and ereptase; analyses of moisture content, ash, total and amino nitrogen, reducing sugars and sucrose; the hydrogen ion concentration, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots; some microchemical and histological observations, and studies conducted during the growth of the plants. As indicated by the title, this paper reports some effects of potassium upon the growth and ash constituents of sugar cane; the following paper deals with the nitrogenous and carbohydrate metabo-

[1] This investigation was conducted in 1931–1932 with the aid of the Sarah Berliner Research Fellowship of the American Association of University Women, at the Experiment Station of the Hawaiian Sugar Planters' Association, and is published at the expense of the Hawaiian Sugar Planters' Association. To both of these organizations the writer desires to express gratitude.

lism and enzyme activity of cane as affected by varying amounts of potassium.

METHODS.—*Saccharum officinarum,* variety H 109, was used in this investigation. This is the most important commercial variety grown in Hawaii at the present time under irrigated conditions. Healthy cane was gathered in the field July 14, 1931, cut to three-eye pieces and soaked in water for one hour. It was then given the hot water treatment (52° C. for 20 minutes) to destroy the stalk mite and to hasten germination. It was

FIG. 1. Arrangement of sugar cane plants at time of transplanting, September 3, 1931.

planted in quartz sand in flats in the greenhouse and watered daily with tap water.

On September 3, 1931, young shoots were cut from the original "seed pieces," or cuttings, with about 1 inch of the cutting remaining attached to each plant, and were planted singly in 5-gallon, glazed, earthenware crocks and were watered with distilled water. The plants were not absolutely uniform at the time of transplanting, varying from 54 to 99 cm.

in length, this measurement being taken to the tip of the longest leaf. Most of the plants had well developed shoot roots. The few which were still dependent upon the seed-piece roots survived transplanting and grew as well as the others. The sand had been cleaned by the method described in the former paper. The pots were covered with overlapping pieces of lacquered cardboard, 4 mm. thick, with a central hole fitting the plant. This hole was enlarged and shaped when necessary to accommodate the developing secondary shoots. Drainage was maintained as described in the former paper. The condition and set-up of the experiment at the time of transplanting, September 3, 1931, are shown in figure 1.

Forty plants were grown in this experiment, eight in each of five different treatments. They were watered with the nutrient solutions for the first time on September 15, 1931. At first each plant received 1 liter of solution twice a week. This amount was increased from time to time, until in April, when the plants were finally harvested, the control plants were each receiving 3 liters every day. Because this amount was generally insufficient for the plants in series 2, there being none left to drain out the following day, these plants were each given 4 liters per day at the end of the experiment. The plants in the other series required less water because of their smaller size. The series of plants were numbered 1 to 5, corresponding to the solutions, in the order of decreasing amounts of potassium. Beginning January 13, 1932, two of the most poorly developed plants in series 4 and 5 were given the control solution regularly in order to study their recovery from potassium starvation. The two plants which were changed from solution 4 to solution 1 were then designated series 6; those changed from solution 5 to the control solution were then called series 7.

The plants were grown in a well ventilated glasshouse and were kept on ant-proofed benches.

The nutrient solutions were a modification of SHIVE'S "best solution" (65) and were prepared from the following stock solutions:

KH_2PO_4	Volume molecular solution
$NaH_2PO_4 \cdot H_2O$	" " "
$Ca(NO_3)_2 \cdot 4H_2O$	" " "
$MgSO_4 \cdot 7H_2O$	" " "
$CaH_4(PO_4)_2 \cdot H_2O$	0.05 Volume molecular solution
$Ca(OH)_2$	Saturated solution

The composition of the nutrient solutions is given in table I.

The reaction of the solution varied from pH 6.3 to 6.5, using chlorphenol red and bromthymol blue as indicators.

Iron was originally added as ferric phosphate but by September 24 some of the plants were beginning to become chlorotic. Washing the

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS, IN CC. PER 2 LITERS

No. of solution	Potassium	Potassium phosphate	Sodium phosphate	Calcium phosphate	Calcium nitrate	Magnesium sulphate	Calcium hydroxide
	p.p.m.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1 (control)	87.9	4.5	.....	.....	10.4	10	50
2	39.0	2.0	2.5	.....	10.4	10	50
3	3.9	0.2	4.3	.....	10.4	10	50
4	0.0	.....	4.5	.....	10.4	10	50
5	0.0	.....	.....	44.8	10.4	10	50

chlorotic leaves with a 5 per cent. solution of ferrous sulphate produced a deeper shade of green within two or three days. Possibly ferric phosphate is not a satisfactory source of iron because it is too insoluble to supply the need of cane. Beginning October 7, 1931, enough iron in the form of ferrous sulphate was added to the sand to keep the plants green.

Since in the latter part of December the plants developed typical symptoms of Pahala blight (described later in this paper), the nutrient solutions were modified in several ways. Beginning January 4, 1932, 0.1 p.p.m. manganese was added to the solutions as $MnSO_4 \cdot 4H_2O$. Beginning March 3, the amount of manganese was doubled in solutions 1 and 2, and on March 23 the quantity was again doubled so that thereafter the plants in series 1 and 2 received 0.4 p.p.m. In addition, some of the leaves were washed with manganous sulphate, others with ferrous sulphate, and finally all of the leaves were dusted with a mixture of manganous sulphate and sulphur. The relative value of these treatments will be discussed later; suffice it to say at this point that by the time of the final harvest, April 27, the symptoms of Pahala blight had practically disappeared.

Because many of the sheaths showed a tendency to rot, this condition becoming apparent early in March, 1932, and because the ash analyses of the plants harvested on November 20, 1931, indicated that the leaves might be deficient in silicon, on March 22 dialyzed silica was added to the solutions. This was added at the rate of approximately 33 p.p.m. Si.

Several staff members of this Station have found that titanium aids in overcoming certain types of chlorosis in sugar cane (unpublished data). Beginning April 4, 1932, titanium nitrate was included in the solutions, at the rate of 10 p.p.m. Ti.

Several sugar cane insects gave trouble during the growing of the plants. These included the armyworm, *Spodoptera mauritia* Boisd.; the cane aphis, *Aphis sacchari* Zehnt.; the gray sugar cane mealybug, *Trionymus boninsis* Kuwana; the pink sugar cane mealybug, *Trionymus sacchari* Ckll.; the stalk mite, *Tarsonemus spinipes* Hirst; and the so-called red spider or leaf mite, *Tetranychus exsiccator* Zehnt. An attempt was made to combat the leaf mite and cane aphis by spraying the plants with distilled water but without success. The plants were thereafter sprayed once a week with Black Leaf 40. This was found to check the insects but not to prevent their development entirely.

COLLECTION AND PREPARATION.—The plant material was harvested at three different intervals, being collected between 10 and 12 o'clock on sunny mornings.

On November 20, 1931, two plants of each of the series were harvested. The tops were cut from the roots, photographed, weighed, and separated into blades, sheaths, and stems. These were chopped separately in an

Athol chopping machine. Duplicate samples of blades and stems to be used for moisture determinations were placed in weighed aluminum boxes, treated with 1 cc. 95 per cent. alcohol, and weighed immediately. Weighed samples of blades and stems for sugar and nitrogen determinations were taken in duplicate, placed in Erlenmeyer flasks, each containing 1 gm. of calcium carbonate (to neutralize acids), and boiled with 100 cc. 95 per cent. alcohol. These samples were stored in the dark until ready for analysis. The remainder of the ground material was spread out between layers of filter paper and dried in a current of air at room temperature. It was then stored in desiccators in the dark. This material was used for enzyme determinations and for ash analyses. The roots were removed from the sand the following day and were dried without being ground.

On December 4, two plants from each of series 1 and 3 were removed from the crocks, the roots included. Photographs and weights were taken. The plants were separated into blades, sheaths, stems, and roots and were ground as just described, the sheaths being discarded. The ground material was placed in large Pyrex test tubes, stoppered, and the tops covered securely with wax paper. They were then frozen in a salt-ice mixture. The juice was expressed and used for determining the titratable acidity, hydrogen ion concentration, and titration curves, the results of which are reported in the second paper of this series.

On April 27, 1932, the final harvest was conducted, which consisted of all the remaining plants. The method was essentially the same as that of the November collection, except that the tops and roots were removed together from the sand. The blades were ground with a Russwin food cutter no. 3. The stems were sliced with a Sterling slicer no. 2 before being ground. As before, the roots were dried without being ground. Samples were taken in the same way as in the November harvest. The material for enzyme and ash analyses was dried in a current of air at a temperature not exceeding 43° C. and generally somewhat lower.

Results

1. Studies during growth

A. Symptoms of potassium deficiency.—The symptoms of potassium starvation which developed in this investigation were depressed growth of the entire plant, discoloration of the leaves, and dieback of the leaf tips. The plants grew rapidly and at first showed no real differences in their growth rates. Watering with the nutrient solutions was started on September 15, 1931. By October 7, *i.e.*, three weeks after starting the plants in the nutrient solutions, a slight gradation in growth was found, corresponding to the amount of potassium supplied, as shown in table II. This

gradation was noticeable in the figures for average size but could not be detected by just glancing at the plants. Growth in length is a measurement taken to the tip of the longest leaf. The total growth in length was determined by adding the growth in length of the main shoot to the total length of the tillers. This gave the total growth in length for the period from September 23 to October 7, because on September 23 the few secondary shoots were all still under the covers. Length measurements were all taken from the top of the cover because of the variation in the depth of the sand.

Differences in colors were hard to detect on October 7. Some of the lower leaves of the plants in series 4 and 5 were a little yellow, but whether this was the first discoloration due to potassium deficiency is hard to say because some of the leaves of series 1 were also slightly yellow.

On October 10 it was noticed for the first time that several of the leaves of the plants of series 3, 4, and 5 had conspicuous red areas on the upper surfaces of the midribs. There was no such discoloration on the midribs of series 1 and 2. The course of the development of red midribs and the histological studies and other investigations dealing with them will be considered later. They are illustrated in the plate.

The yellow condition of the lower leaves of the potassium-deficient plants became more pronounced. Gradually the edges of the leaves turned brown and died. This was accompanied by the dying and curling of the

TABLE II

MEASUREMENTS OF PLANTS TAKEN OCTOBER 7, 1931, THREE WEEKS AFTER WATERING WITH NUTRIENT SOLUTIONS WAS BEGUN

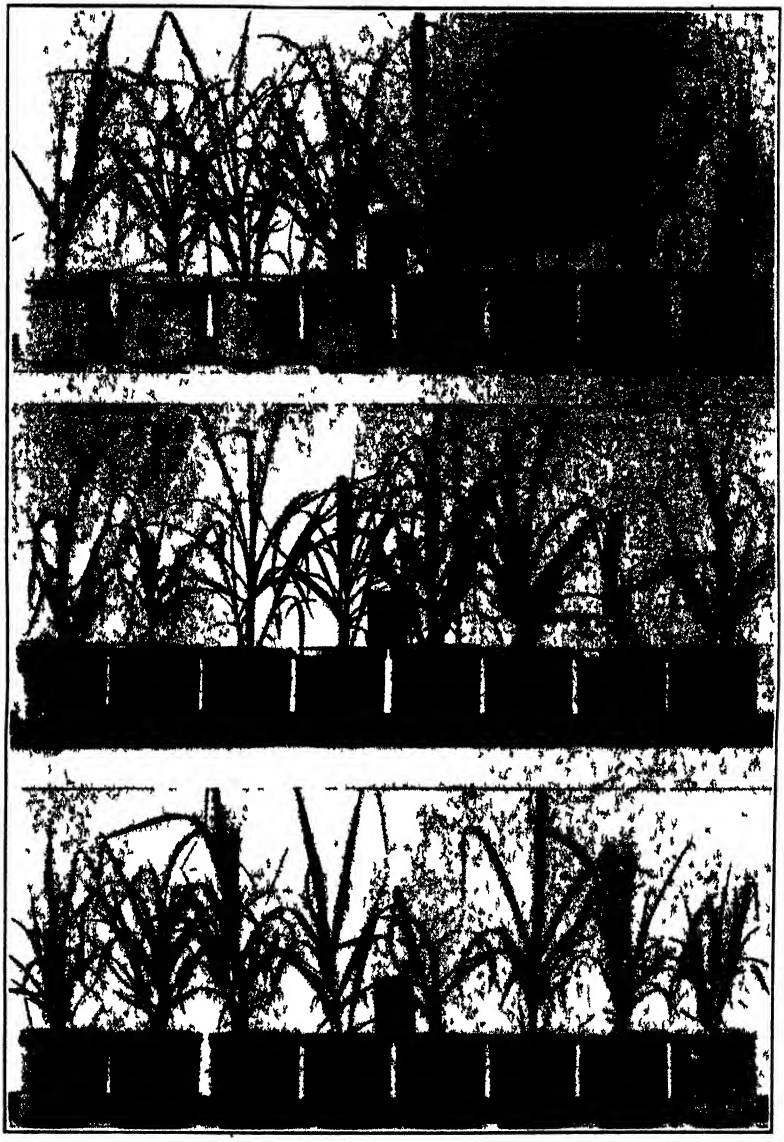
SERIES	AVERAGE GROWTH IN LENGTH OF MAIN SHOOTS, SEPT. 23 TO OCT. 7	AVERAGE TOTAL LENGTH OF TILLERS, OCT. 7	AVERAGE TOTAL GROWTH IN LENGTH	AVERAGE NUMBER OF TILLERS
	cm.	*cm.*	*cm.*	
1	32.06	61.1	93.16	2.37
2	37.02	63.9	100.92	2.25
3	28.6	53.18	81.78	2.5
4	25.4	44.7	70.1	2.25
5	25.4	38.0	63.4	2.0

tips of the leaves, a condition known as dieback. Small red and red-brown spots developed in the laminae of the blades of the plants deficient in potassium. These symptoms were most fully developed in the plants of series 4 and 5, between which no difference could be observed. At first they were

not so conspicuous in series 3 as in 4 and 5; they never developed in series 1, and occurred only slightly in series 2 toward the end of the experiment. By October 23, the plants of series 3, 4, and 5 showed definite symptoms, including decreased growth, discoloration, and dieback. Figures 2 to

FIGS. 2 and 3. Series 1 (controls), on October 27, 1931, six weeks after starting them in the nutrient solutions. Series 2 on October 27, 1931.

6 show the plants on October 27, six weeks after starting them in the nutrient solutions. A definite gradation in size will be noted, as will also the similarity of the plants of series 4 and 5.



FIGS. 4–6. Series 3 (above) on October 27, 1931. Series 4 (middle) on October 27, 1931. Series 5 (below) on October 27, 1931.

The plate shows the typical colors of the plants. The sketches were made by a Station artist November 5 and 6, 1931, in the greenhouse from the living leaves. *A* shows a typical leaf from a control plant. It will be noted that there are some very small light green flecks on the lamina Whether these are due to insect injury or are a similar condition (only less devel-

oped) to the red spots on the plants deficient in potassium was not ascertained. Histological studies by WELLER of this Station showed no mycelium. *B*, *C*, and *D* show the typical colors of the plants deficient in potassium; light, yellow-green laminae, yellow-brown edges, red midribs, red flecks in the laminae, and dieback. *B* shows the color of a midrib soon after it has become red, while *C* shows the darkening of the discoloration as the leaf ages. More will be said later regarding which of these may be considered primary and which secondary symptoms of potassium deficiency.

Symptoms of potassium deficiency under field conditions are the same as those already described. Similar symptoms also developed in the former investigation (**28**), with the exception of the red midribs. The yellow discoloration between the veins of the upper leaves occurring in the Chicago studies was probably due to a slight development of Pahala blight rather than to potassium deficiency. It would seem now that the symptoms of potassium deficiency in sugar cane are well established, comprising decreased growth of the entire plant, dieback of the leaf tips, and the discolorations of the blades as shown in the color plate. The brown edges and tips of the blades and yellowish green color of the laminae are primary symptoms while, as will be shown later, the red discoloration of the midribs is considered secondary.

The first harvest, consisting of two plants from each series, was conducted November 20, *i.e.*, about nine weeks after watering with the nutrient solutions was started. Averages of the measurements of the plants taken on November 19 are presented in table III. "Dewlap" is a term commonly applied in sugar cane parlance to the joint between the blade and the sheath. The height of the highest emerged dewlap is considered the best measurement of the growth of cane. According to VAN DEVENTER (**69**),

TABLE III

MEASUREMENTS TAKEN NOVEMBER 19, NINE WEEKS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS, IN AVERAGES

SERIES	LENGTH OF MAIN SHOOTS	TOTAL LENGTH OF TILLERS	HIGHEST DEWLAP	BREADTH OF LONGEST LEAF	NO. OF TILLERS	NO. OF RED MIDRIBS
	cm.	*cm.*	*cm.*	*cm.*		
1	189.4	566.7	38.6	4.1	12.0	0.0
2	192.6	787.7	36.7	3.9	13.0	6.7
3	163.9	332.9	25.5	3.1	2.7	17.0
4	140.2	181.0	20.1	2.7	2.2	10.5
5	145.1	243.7	20.3	2.7	2.5	19.7

the highest leaf joint is about 30 cm. above the real vegetative point because the sheath of that leaf is fully grown, or nearly so. Breadth of leaf is a measurement taken at the broadest part of the longest leaf; this is generally about the middle of the leaf. The average growth of the plants for the period November 3 to 19 is given in table IV. Fresh weights of blades, sheaths, and stems, and air-dry weights of roots of the plants collected November 20 are given in table V. Tables III to V show that nine weeks after starting the plants in the nutrient solutions, the plants of series 1 and 2 were considerably larger in every measurement than the plants starved for potassium, series 3 to 5.

TABLE IV

AVERAGE GROWTH, NOVEMBER 3 TO 19

SERIES	LENGTH OF MAIN SHOOTS	HIGHEST DEWLAP	LENGTH OF TILLERS	NO. OF TILLERS
	cm.	*cm.*	*cm.*	
1	15.4	7.7	262.6	5.5
2	17.5	5.0	464.2	5.0
3	14.9	2.1	73.8	Decreased in number
4	4.9	0.0	0.0	"
5	12.7	0.2	55.7	"

TABLE V

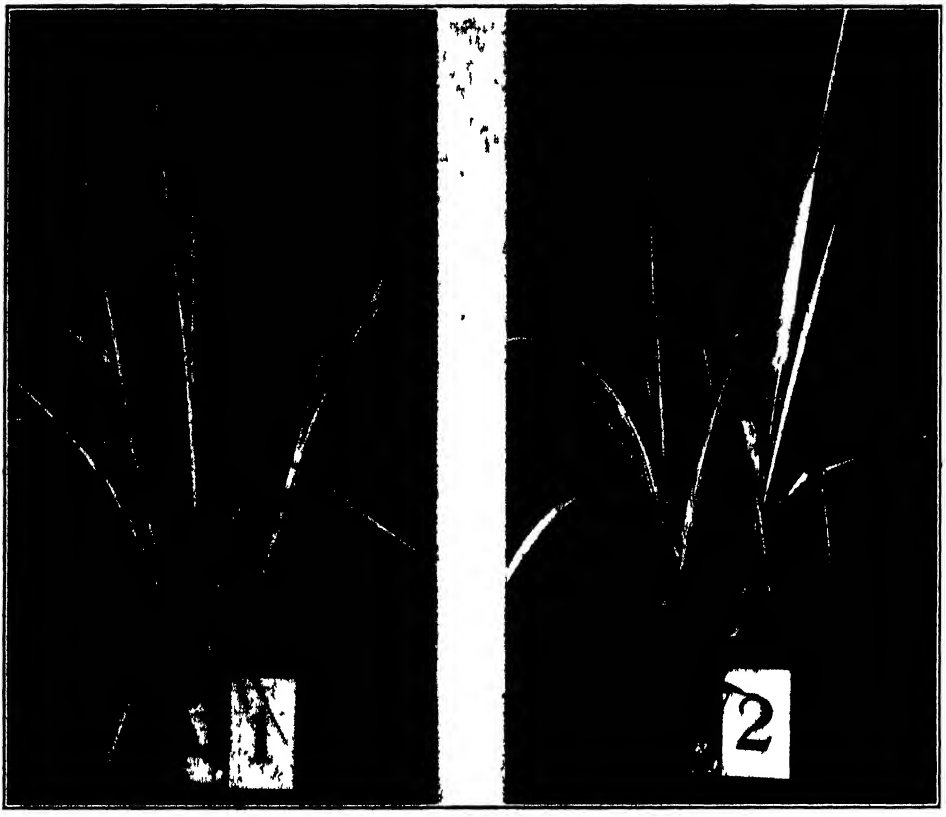
AVERAGE WEIGHTS OF PLANTS, NOVEMBER 20

SERIES	FRESH WEIGHT				AIR-DRY WEIGHT
	BLADES	SHEATHS	STEMS	TOTAL TOPS	ROOTS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	256.0	188.0	81.0	526.0	23.7
2	269.2	196.0	86.2	551.0	28.7
3	130.0	71.2	24.0	225.2	11.2
4	68.0	30.0	9.2	107.7	5.8
5	78.7	35.0	14.0	128.2	6.0

In most measurements the plants of series 2 averaged larger than those of series 1. Attention is drawn to the close similarity between the plants of series 4 and 5; where there is a difference between them it is in favor of series 5. Evidently sodium does not replace potassium in the growth of

sugar cane. Figures 7 to 11 show the plants at the time of harvesting, November 20, 1931.

Besides the colors, which are shown in the plate, several other differences should be mentioned. There was a gradation in the development of wax on the stems correlated with the supply of potassium. The roots showed a



FIGS. 7 and 8. Typical plant (left) of series 1 (control) on November 20, 1931, nine weeks after starting the plants in the nutrient solutions. Typical plant (right) of series 2 on November 20, 1931.

gradation in size and condition, those of series 1 and 2 being large, firm, and white and those of the plants deficient in potash being small, poorly developed, and discolored yellow and brown.

The second harvest was conducted December 4, eleven weeks after starting the plants in the nutrient solutions. At this time two plants from each of series 1 and 3 were collected. Table VI gives the average measurements of all the plants taken December 3. Table VII gives the weights of the tops and roots of the plants harvested December 4, 1931.

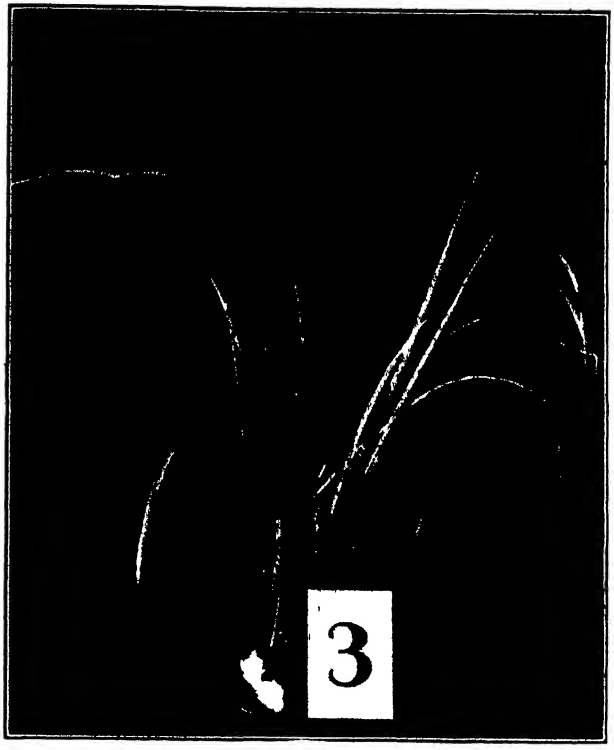


FIG. 9. Typical plant of series 3 on November 20, 1931.

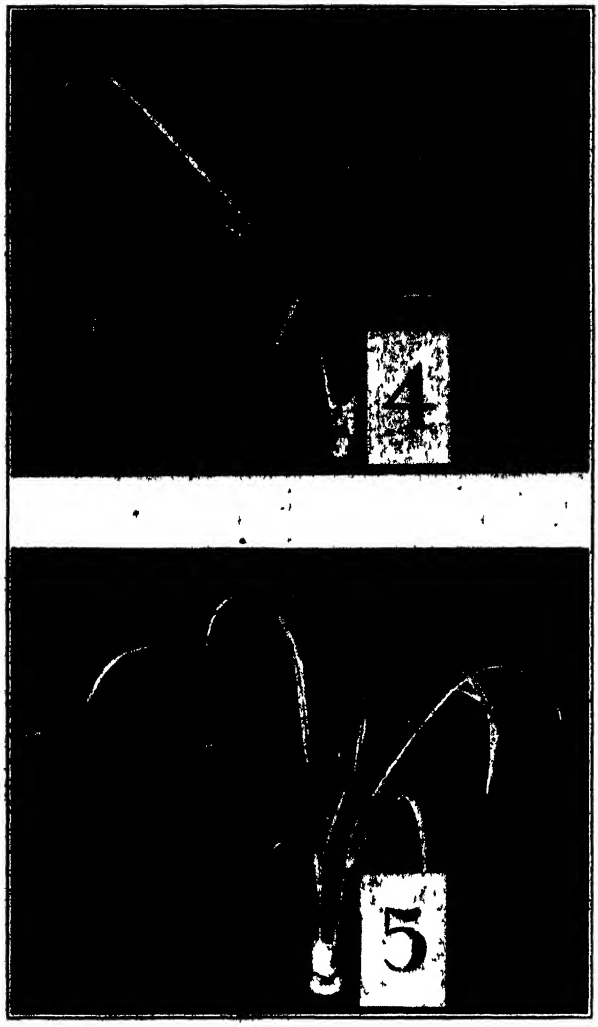
Figures 12 and 13 illustrate the plants just after they were removed from the crocks.

On January 13, about four months after starting the plants in the nutrient solutions, two of the most poorly developed plants of series 4 and 5 were

TABLE VI

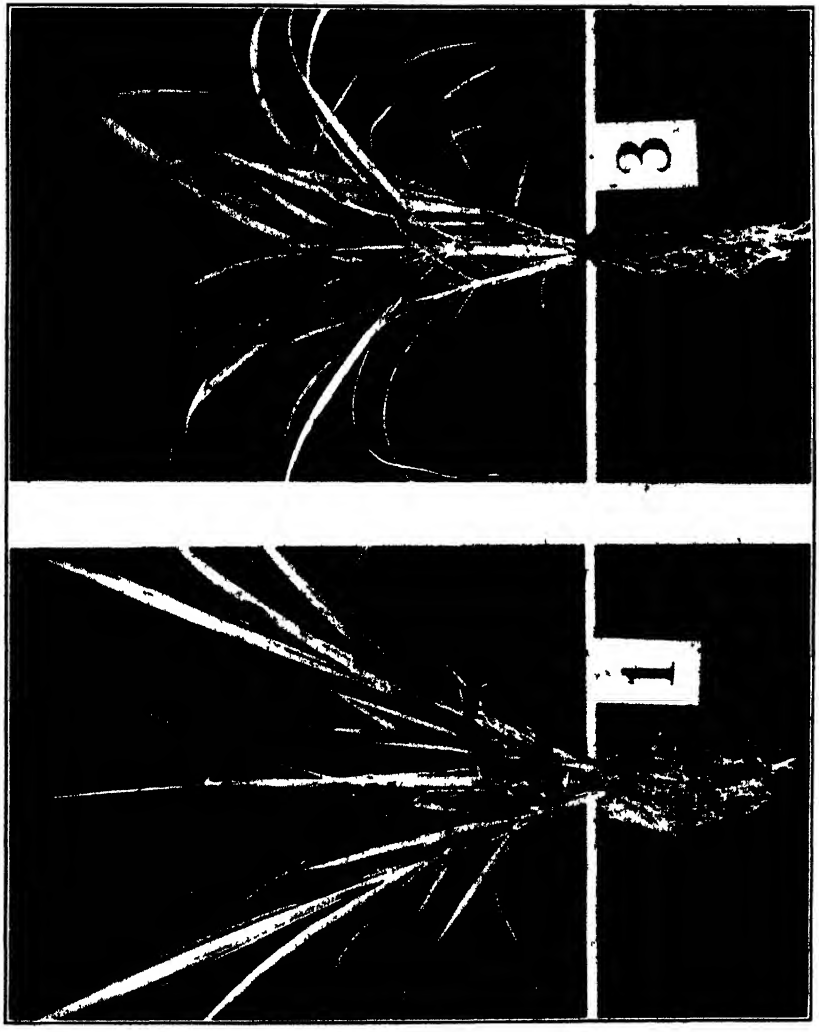
AVERAGE SIZE OF PLANTS, DECEMBER 3, ELEVEN WEEKS AFTER STARTING SOLUTIONS

SERIES	LENGTH OF MAIN SHOOTS	BREADTH OF LONGEST LEAF	HIGHEST DEWLAP	NO. OF TILLERS
	cm.	*cm.*	*cm.*	
1	196.1	4.5	47.2	11.5
2	198.9	4.2	42.5	12.5
3	165.0	3.0	25.8	4.5
4	143.5	2.5	21.3	3.0
5	150.1	2.7	22.3	2.5



FIGS. 10 and 11. Typical plant (above) of series 4 on November 20, 1931. Typical plant (below) of series 5 on November 20, 1931.

given solution 1, this treatment being continued to the end of the experiment. Their appearance on January 13, before starting the new treatment, is shown in figure 14. Thereafter these were called series 6 and 7 respectively. They immediately started to grow. The stems which were already formed became more rigid, straightened up, lengthened, and increased in circumference. Adventitious roots developed at the base of the stem, which acted like prop roots in supporting the rapidly developing stems. Buds also developed at the bases of the stems, and quickly formed new shoots.



FIGS. 12 and 13. Typical plant (left) of series 1 on December 4, 1931. Typical plant (right) of series 3 on December 4, 1931.

The old leaves, which exhibited the symptoms of potash deficiency, dried up, died and fell down; the newly developed leaves were as green and nearly as large as those of the plants of series 1. The increased growth of the stems is shown graphically in figure 15. By the latter part of February, these plants had overtaken and surpassed the plants of series 3 in development. It is interesting that series 7 showed a greater capacity for recovery than did series 6. Their measurements at the time of the final

TABLE VII

WEIGHTS OF TOPS AND ROOTS, DECEMBER 4

PLANT	TOPS	ROOTS
	gm.	*gm.*
1 G	800.0	448.0
1 H	885.0	354.9
3 G	222.0	201 0
3 H	265.5	246.0

harvest, April 27, are given in tables VIII and IX; their appearance at that time is shown in figures 21, 22, 28, and 29.

The final harvest of all the plants was conducted April 27, seven and one-half months after starting the plants in the nutrient solutions. Size measurements taken on April 25 are presented in table VIII. The fresh weights of the tops collected April 27 and the air-dry weights of the roots are given in table IX. Figures 16–22 show all the plants on April 25.

FIG. 14. Appearance of series 4 and 5 on January 13, 1932, just before changing to solution 1. Thereafter these plants were called series 6 and 7 respectively.

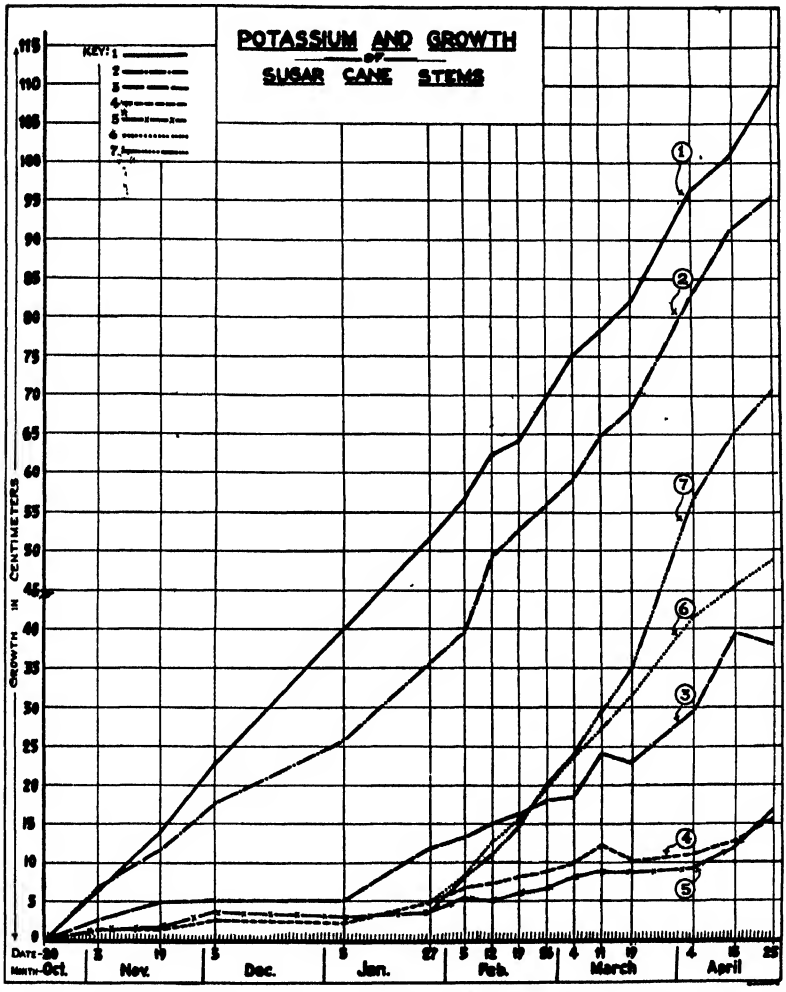
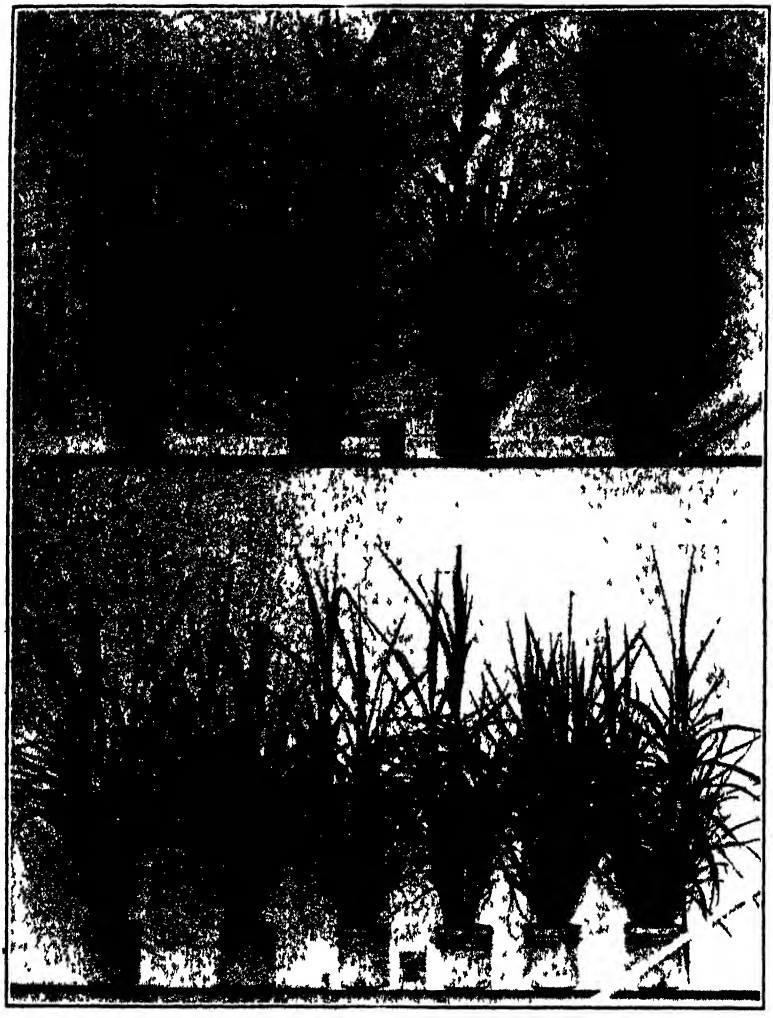


FIG. 15. Effect of potassium upon growth of stems as indicated by the average height of the highest emerged "dewlap" (juncture of blade and sheath).

Photographs of representative plants taken at the final harvest are shown in figures 23–29. The colors of the plants were practically the same as before, except that some of the leaves of the plants of series 1 and 2 were burned, owing to the high temperature in the greenhouse. The plants showed very little Pahala blight, these symptoms being confined chiefly to the old leaves of the secondary shoots. Most of the plants deficient in potassium had red midribs. The plants of series 6 and 7 had the best developed individual roots, because they were not yet potbound. Some of the longer roots showed plainly where they had begun to increase growth

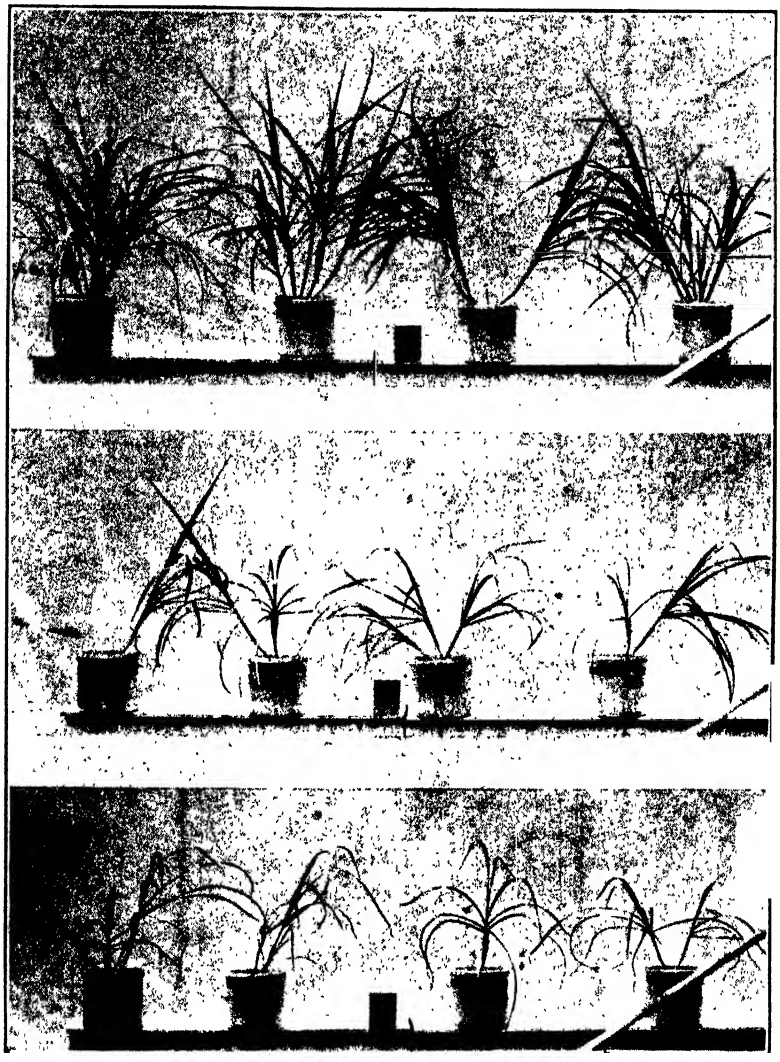


FIGS. 16 and 17. Series 1 (above) on April 25, 1932, 7½ months after starting them in the nutrient solutions. Series 2 (below) on April 25, 1932.

at the time of the first addition of potassium. The plants of series 1 and 2 had very large and interwoven masses of roots.

Graphs of the increase in height of the highest emerged dewlap are given in figure 15. It will be noted that the growth of the stems is markedly affected by the amount of potassium supplied. After the first week or so the plants of series 1 maintained a steady growth, always greater than that of any of the others. The speedy recovery of the plants of series 6 and 7 after the addition of potassium in January is conspicuous. The

increase in growth of series 3, 4, and 5 beginning in January may be due in part to the increased light in the spring, but is probably due also to the fact that manganese was added to the solutions for the first time on January 4.

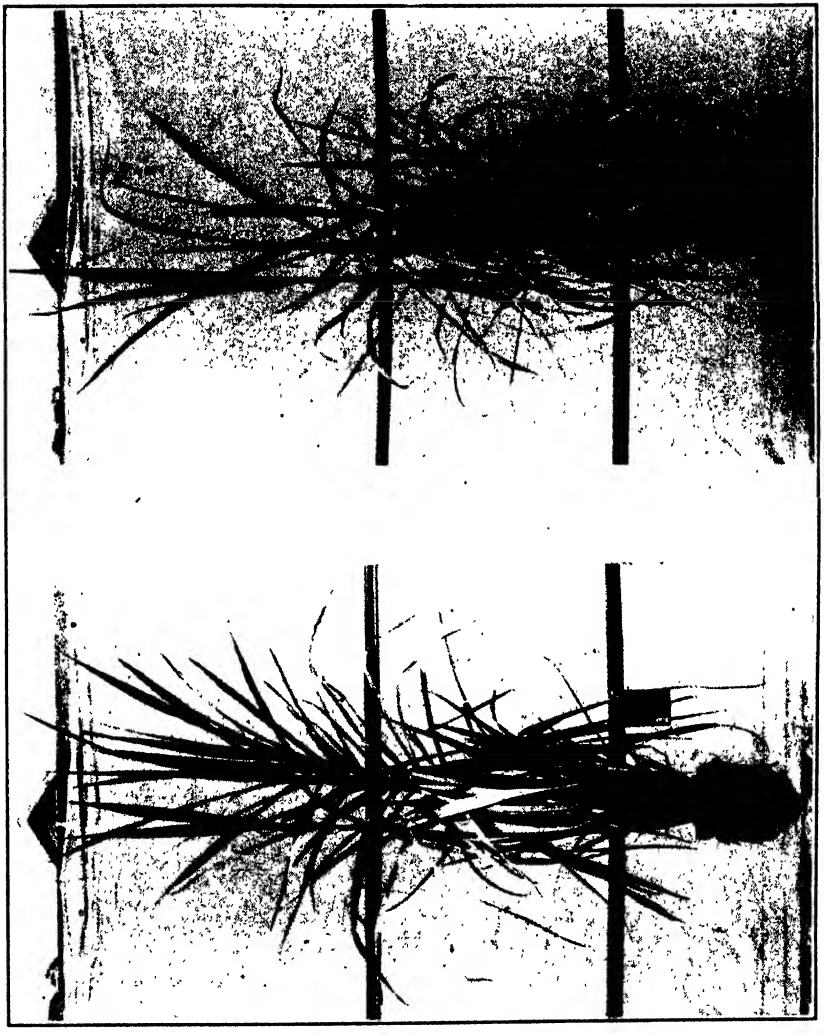


FIGS. 18–20. Series 3 (above) on April 25, 1932. Series 4 (middle) on April 25, 1932. Series 5 (below) on April 25, 1932.

That manganese is essential for the growth of sugar cane has been shown by DAVIS (15) and MARTIN (unpublished work). The development of Pahala blight in the absence of manganese has been studied by LEE and MCHARGUE (45) and by MARTIN (48).

FIGS. 21 and 22. Series 6 (above) on April 25, 1932. Until January 13, these plants received solution 4; after that time, solution 1. Series 7 (below) on April 25, 1932. Until January 13, these plants received solution 5; after that time, solution 1.

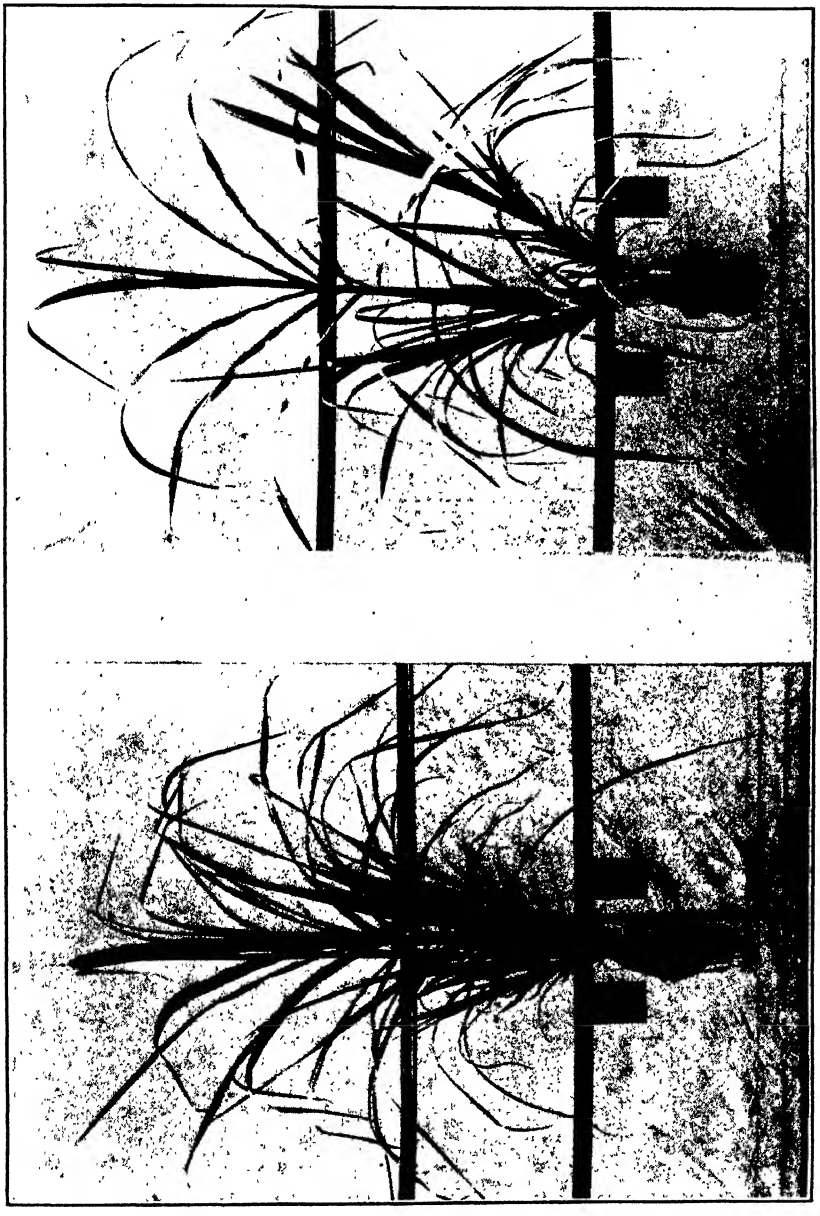
Mention has already been made of the development of red midribs on the leaves of plants deficient in potassium. The average numbers of red midribs per plant are shown in table X. These counts were discontinued after November 22. No red midribs developed in the plants of series 1. In



FIGS. 23 and 24. Typical plant (left) of series 1 at the final harvest, April 27, 1932. Typical plant (right) of series 2 on April 27, 1932.

FIGS. 25–27. Typical plant (above) of series 3 on April 27, 1932. Typical plant (below, left) of series 4 on April 27, 1932. Typical plant (below, right) of series 5 on April 27, 1932

general, they occurred on most of the plants of series 3, 4, and 5 up to the end of the experiment. It will be seen that their occurrence is correlated with a deficiency in potash. The question arose as to whether this discoloration is a specific symptom of potash deficiency in cane or a secondary reaction.



FIGS. 28 and 29. Typical plant (left) of series 6 on April 27, 1932. Typical plant (right) of series 7 on April 27, 1932.

TABLE VIII

AVERAGE SIZE OF PLANTS AT FINAL HARVEST, APRIL 25, 7½ MONTHS AFTER STARTING IN THE NUTRIENT SOLUTIONS

SERIES	HIGHEST DEWLAP OF MAIN STALKS	BREADTH OF LEAF AT MIDDLE OF LONGEST LEAF OF MAIN STALK	CIRCUMFERENCE OF MAIN STALKS
	cm.	*cm.*	*cm.*
1	130.9	5.5	9.3
2	116.4	4.4	8.4
3	57.9	3.9	5.5
4	34.3	2.6	3.8
5	36.0	2.6	3.7
6	69.0	4.9	7.7
7	94.3	5.6	9.3

Red midribs frequently occur on cane grown in the field resulting from infection by the red rot fungus, *Colletotrichum falcatum*. This fungus gains entrance through mechanical injuries or following leafhopper injury. The plants of the present investigation were examined by the pathologists and entomologists of this Station and no leafhopper punctures were found. As reported by MARTIN (47), samples studied histologically by WELLER of this Station showed no mycelium. Attempts made by MARTIN to isolate an

TABLE IX

AVERAGE WEIGHTS OF PLANTS COLLECTED APRIL 27, EXPRESSED IN GRAMS

SERIES	FRESH WEIGHT				AIR-DRY ROOTS
	BLADES	STEMS	SHEATHS	TOTAL TOPS	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	955.88	2185.10	1009.75	4150.73	108.5
2	1035.30	1639.50	993.60	3668.40	106.8
3	277.70	176.95	172.92	627.57	27.8
4	62.02	25.20	28.30	115.52	8.2
5	63.70	28.05	31.87	123.62	11.3
6	569.75	358.25	443.00	1371.00	47.6
7	472.50	415.25	337.00	1224.75	40.5

TABLE X

AVERAGE NUMBER OF RED MIDRIBS

	Series				
	1	2	3	4	5
October 15	0	0	1.5	2.3	1.5
October 20	0	0	4.8	4.7	5.6
November 4	0	0.6	12.7	12.6	11.8
November 22	0	6.7	17.0	10.5	19.7

organism from red-midrib tissue were negative. Hypodermic injections of 0.25 per cent. acetic acid into the midribs of other cane plants did not result in the development of any red discoloration. It was concluded that the red midribs were not caused by an organism or by acidity alone.

Microscopic studies showed that the red color of the midribs was a discoloration of the lignified cell walls. The cuticle and epidermis were not affected. The walls of the subepidermal plates were red, also the sheaths of the large vascular bundles, the xylem, and the lignified regions between the bundles and the lower epidermis. The color of the walls was the same as that which develops in the microchemical test for lignin, *viz.*, staining with phloroglucin-hydrochloric acid. The course of the discoloration seemed to be as follows: first the protoplasm of the parenchyma became slightly brown, then the parenchyma walls next to the lignin became faint red, finally the lignified walls reddened. Thus the disturbance seemed to originate in the parenchyma.

STEUERWALD (**67**) isolated saccharetin from cane fiber and stated that it belongs to the phlobaphenes and may be considered the aromatic compound in lignin. It is very soluble in alcohol. On treatment with phloroglucin-hydrochloric acid, saccharetin becomes intensely red.

Because of these characteristics of saccharetin and because of the resemblance of the discolored walls to the ordinary lignin test, it was thought that phloroglucin might be present in the cells of the potassium-deficient leaves, and thus explain their discoloration. Phloroglucin occurs in some plants as a hydrolytic product of certain tannic acids (**23**).

Evidence of the presence of phloroglucin was obtained as follows. Heating sections of the discolored midribs in alcohol partially removed the red color. When a partially decolorized section was treated with concentrated hydrochloric acid, with no addition of phloroglucin, the reddish color deepened. Subsequently heating caused a decided reddening of the walls. When sections of the control leaves were heated with hydrochloric acid no

suggestion of red occurred, although decisive tests for lignin were obtained using phloroglucin-hydrochloric acid. It would seem that the plants of series 1 contained no phloroglucin, while the potassium-deficient plants did.

The need for an acid as well as phloroglucin for the red coloration of saccharetin was realized. The presence of enough hydrochloric acid within the plants was doubted, since the nutrient solutions contained no chlorides. The attempt was made to produce a similar color using tannic acid instead of hydrochloric, but without success. Sulphuric acid, however, gave a red color very similar to that formed when hydrochloric acid was used, and closely resembling the discolored midribs, both in shade and location. The first red midribs to develop were noticed on October 10, just three days after the first application of ferrous sulphate in the nutrient solutions. It would appear that this offers an explanation of the red midribs of the potassium-deficient plants.

To sum up, it seems possible that potassium deficiency causes a decomposition of tannic acids, resulting in the formation of phloroglucin. This in the presence of an excess of sulphuric acid may cause the reddening of saccharetin. The development of red midribs is thus a secondary rather than a primary symptom of potassium deficiency, and will occur only in the presence of an excess of sulphates or possibly chlorides.

Since the above was written, confirmatory evidence of the importance of sulphates in the development of red midribs has been supplied by W. W. G. Moir, of American Factors, Honolulu, who states that at Olaa Sugar Company red midribs developed within a month after the application of two tons of sulphur per acre to land deficient in potassium. The midribs of other plants became red after the application of copper sulphate.

In this connection it is interesting to note that it is characteristic of sugar cane to become reddened when diseased or injured, whatever the causal agent. Although other factors are probably involved also, the possibility is suggested that wherever a red color develops in the cell walls, following disease or injury, it may be due to the action of sulphates or chlorides and phloroglucin upon saccharetin. Sugar cane soils are often high in sulphates, and it seems possible that many diseases might lead to the decomposition of tannic acids and thus the formation of phloroglucin.

Van Den Honert (70) recently described symptoms of potassium deficiency obtained with variety P. O. J. 2878, which in general agree with those described here. The main difference between the Java results and ours lies in the location of the discoloration of the midribs. Van Den Honert gives figures of cross sections of the discolored midribs and states that in potassium deficiency the discoloration is confined to the upper surface; whereas all the lignified regions of the midribs of the potassium-deficient plants appeared red in places in the investigation herein reported,

when examined microscopically. It is interesting to note that VAN DEN HONERT used sulphuric acid in his nutrient solutions, because of the importance of sulphates in the production of red midribs brought out in this study. The gumming of the epidermal walls of the midribs considered a symptom of potassium starvation by VAN DEN HONERT was not observed in the present study.

B. ABSORPTION OF POTASSIUM.—Three experiments were conducted dealing with the absorption of potassium. On October 16, composite samples of the drainage of the plants in series 1 to 3 were collected. Each plant had received 1.5 liters of solution four days previously. Analyses for potassium were performed by the Chemistry Department of this Station and the results are given in table XI. The control solution prepared that week contained 92 p.p.m. potassium, by analysis. Analyses of the other solutions were not made, the calculated parts-per-million being used to obtain the amount of potassium absorbed by the plants of series 2 and 3. It will be noted that the plants of series 1 and 2 absorbed potassium at the same rate that week.

TABLE XI

POTASSIUM ANALYSES OF DRAINAGE COLLECTED OCTOBER 16, 1931

SERIES	K IN DRAINAGE	K ABSORBED IN 4 DAYS
	p.p.m.	*p.p.m.*
1	62.0	30.0
2	9.4	30.0
3	2.1	1.8

Several investigators have found evidence of a relationship between the absorption and utilization of potassium and light, which will be mentioned more completely in the discussion. To determine whether the amount of potassium absorbed during the day differs from that taken up by night, the following test was performed. On November 13 and 14, drainage samples were collected from the plants of series 1 and 2 at 6 A. M. and 6 P. M. Fresh solutions were added each time. The samples were analyzed for potassium by the Chemistry Department of this Station. Sunrise occurred at 6:11 and sunset at 5:20, according to the Weather Bureau. It was therefore possible to determine the amount of potassium absorbed during approximately 12 hours of darkness and 12 hours of daylight. The results are reported in table XII, which shows that both sets of plants absorbed the same amount of potassium during the night and the day.

TABLE XII

DAY AND NIGHT ABSORPTION OF POTASSIUM

SERIES	WATERED 6 P. M. FRIDAY AND 6 A. M. SATURDAY	CONTAINED 6 A. M. SATURDAY	CONTAINED 6 P. M. SATURDAY	ABSORBED IN 12 HOURS' DARKNESS	ABSORBED IN 12 HOURS' DAYLIGHT
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	93.6	4.7	5.2	89.9	88.4
2	43.1	3.0	3.5	40.1	39.6

The object of the third experiment dealing with absorption was to determine whether or not cane plants can absorb potassium through their leaves. It is well known that iron can be absorbed that way. Evidence has been presented by SHAW (**63**) showing that cane leaves may absorb large quantities of water during rains. DOMONTOVICH and ZHELEZNOV (**17**) have shown that painting leaves with salts of potassium and magnesium leads tc the absorption of these elements.

On March 18, several leaves of the plants of series 5 were washed with a 5 per cent. solution of potassium dihydrogen phosphate. No sign of recoloration was observed. The results of this test are inconclusive, since it is possible that potassium might be absorbed without causing the leaves to become green. On the other hand, it may be that they were already beyond recovery.

C. HYDROGEN ION CONCENTRATION OF DRAINAGE.—Colorimetric determinations of the hydrogen ion concentration of the drainage were made at intervals. The reaction of the nutrient solutions was pH 6.4 and the object of these tests was to ascertain how well this reaction was maintained. The results are presented in table XIII. The high acidity was probably due to

TABLE XIII

pH OF DRAINAGE

SERIES	COMPOSITE DRAINAGE					INDIVIDUAL	
	OCT. 23	OCT. 26	OCT. 30	NOV. 2	NOV. 6	NOV. 9	NOV. 11
1	3.4	3.3	3.9	3.9	4.4	6.5–7	5.9–7.1
2	3.6	3.7	4.6	6.1	5.6	7	6.9
3	3.6	4.2	4.3	4.1	4.7	3.9–6.1	.....
4	3.7	2.8	3.7	3.9	4.3	3.5–6.1	.....
5	3.5	2.8	3.5	3.7	4.0	3.6–5.9	.....

the use of ferrous sulphate as the source of iron. Because of the acidity, the solutions were changed more frequently. An important fact is that there was as much variation in reaction between the individuals of one series as there was between the different series, indicating that the differences in growth and external appearance of the plants were not caused by differences in the reaction of the medium. There was no correlation within a series between hydrogen ion concentration and general appearance, length of leaf, height of dewlap, number of red midribs, or amount of dieback. McGeorge (**51**) found that a hydrogen ion concentration as acid as pH 4.0 was without effect upon the growth of cane. An important conclusion to be drawn from these tests is that cane can grow in quartz sand cultures at a reaction varying from about pH 3.5 to 7 without any apparent external differences. In the field the range is probably less than that, since under field conditions changes in hydrogen ion concentration would cause more or less aluminum to go into solution, which would have a decided effect upon growth, as mentioned by Moir at the 52d annual meeting of the Hawaiian Sugar Planters' Association, December, 1932.

D. Pahala blight.—The symptoms of Pahala blight which began to develop the latter part of December, 1931, were alternating stripes of green and white in the blades, small elongated red spots in the white stripes, longitudinal splitting of the blades, and cessation of growth of the secondary stems. These symptoms are the same as those described by Lee and McHargue (**45**). They were confined to the plants of series 1 and 2.

The addition of 0.1 p.p.m. manganese as manganous sulphate to the nutrient solutions, begun January 4, resulted in a diminution of the symptoms of the blight. The secondary shoots started growing again. Because of a recurrence of the symptoms, the amount of manganese in solutions 1 and 2 was doubled twice in March.

Washing the leaves with 1 and 3 per cent. solutions of ferrous sulphate and manganous sulphate on March 4 and 19 resulted in no recoloration of the leaves. On March 21, plant 1 E was dusted with a mixture composed of equal quantities of manganous sulphate and sulphur. In two days this plant looked better. Thereafter all the plants of series 1 and 2 were dusted once a week. By the time of the final harvest the symptoms of Pahala blight had practically disappeared.

E. Silicon and titanium.—As mentioned earlier, silicon was added to the solutions beginning March 22, 1932. This was deemed necessary because of the rotting of the sheaths. On March 28 it was noted that the sheaths, particularly those of the potassium-deficient plants, were stronger, stiffer, greener, and less inclined to rot. Early in April it was noted that none of the plants had as many dead leaves as before the use of silicon. Although the addition of silicon was not a controlled experiment, the evi-

dence presented here seems to indicate that it is essential for the best growth of cane.

There was no evidence that the addition of titanium nitrate to the solutions produced any effect upon the growth or appearance of the plants.

2. Microchemical observations

The stems of the potassium-deficient plants were found to contain iron at the nodes when given the Hoffer test (33), while the stems of the controls showed very little accumulation of iron. A dark discoloration of the bundles of the stems of the plants deficient in potassium was noted before staining. The sieve tubes and companion cells of some of the bundles of the midribs of the plants of series 5 were found to be very dark brown, when examined October 29, 1931. These discolorations were not found in the control plants.

3. Analytical data

The results of the moisture determinations are found in table XIV. In general, the plants supplied with potassium had a higher percentage of water than the potassium-deficient plants.

TABLE XIV

Moisture percentages in cane

Series	November harvest		April harvest	
	Blades	Stems	Blades	Stems
	%	%	%	%
1	74.1 ± 0.071	83.4 ± 0.047	79.1 ± 0.333	86.35 ± 0.071
2	74.9	85.6 ± 0.023	74.65 ± 0.071	83.65 ± 0.548
3	72.1 ± 0.238	81.0 ± 0.381	75.3 ± 1.09	79.7 ± 0.095
4	.....	.....	76.85 ± 0.071	80.75 ± 0.071
5	.....	.....	76.85 ± 0.548	76.75 ± 0.023
6	.....	.....	78.15 ± 0.023	.....
7	.....	.....	77.65 ± 0.071	.....

The results of the ash analyses, which were performed by the Chemistry Department of this Station, are given in tables XV and XVI, in which they are expressed on the percentage basis. The percentage of potassium within the plants was directly proportional to the amount supplied in the nutrient solution. In November the percentage of total ash was higher in the plants deficient in potassium than in the controls, in both blades and roots, whereas in April the blades of the control plants had the highest percentage of ash, the amount in the roots remaining (as before) greater in the potassium-deficient plants. In November the blades of the plants deprived of potash

had the highest percentages of phosphorus, calcium, and magnesium; in the roots, phosphorus was greater in the potassium-deficient plants, but calcium and magnesium did not vary greatly. Silicon varied inversely with the amount of potassium in the blades, while in the roots the percentage of silicon varied irregularly, in the November material. While the high percentages of silicon in the roots in both the November and April material might suggest the possibility of contamination with minute particles of quartz sand, yet every precaution was taken to prevent such contamination and the chemist stated that no grains of sand were found in the ash.

In April the percentage of potassium in the blades, stems, dead leaves, and roots varied directly with the amount supplied. It is interesting that

TABLE XV

ASH ANALYSIS OF PLANTS HARVESTED NOVEMBER 20, 1931

PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	ASH	SI	K	P	CA	MG
	%	%	%	%	%	%
Blades						
1	5.139	0.0659	1.78	0.359	0.279	0.170
2	4.222	0.0617	0.955	0.396	0.411	0.384
3	4.666	0.0892	0.540	0.668	0.502	0.540
4	5.636	0.121	0.424	0.882	0.503	0.645
5	5.917	0.100	0.458	0.983	0.557	0.521
Roots						
1	24.96	8.008	1.78	0.598	0.532	0.308
2	17.42	4.555	0.741	0.450	0.765	0.471
3	28.56	9.395	0.388	0.747	0.515	0.274
4	31.74	11.42	0.294	0.721	0.550	0.237
5	33.82	10.99	0.329	0.856	0.616	0.268

the plants of series 7 contained a higher percentage of potassium in the blades, stems, and roots than the controls. The percentage of potassium in series 6 also surpassed that in series 1 in stems and roots. Evidently the plants which received the first application of potassium in January absorbed it at a high rate.

In April phosphorus was again found to be higher in the potassium-deficient plants, in general. The percentage of silicon was inversely proportional to the potassium content in the roots, in the April material. Calcium, which was higher in the potassium-deficient plants in November, showed the opposite relationship in the blades in April but no striking differences in the other organs. Magnesium was higher in the blades and stems of the potassium-deficient plants than in those of the controls.

The plants receiving sodium contained more of that element than the other plants, but except in the stems the percentage of sodium in the plant was not proportional to the amount supplied in the nutrient solutions. In general the percentages of sodium decreased from the roots to the stems to the blades, whereas the percentages of potassium increased. All the blades and the stems contained higher percentages of potassium than sodium, even those supplied with more sodium than potassium. With the exception of the plants of series 3 and 5 the same condition existed in the roots.

There were greater percentages of iron in the blades, stems, and roots of the plants deficient in potassium than in the controls. The amount of

TABLE XVI

ASH ANALYSIS OF PLANTS HARVESTED APRIL 27, 1932

PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	ASH	SI	K	P	CA	MG	NA	FE
	%	%	%	%	%	%	%	%
Blades								
1	7.34	0.118	2.175	0.5102	0.544	0.4301	0.014	0.002
2	7.88	0.094	1.801	0.5341	0.842	0.5621	0.037	0.017
3	5.61	0.103	0.724	0.6529	0.278	0.5163	0.030	0.054
4	6.46	0.130	0.355	0.8007	0.215	0.6379	0.033	0.031
5	6.41	0.174	0.501	0.7893	0.241	0.5412	0.026	0.052
6	6.47	0.151	1.765	0.5269	0.279	0.2717	0.025	0.031
7	6.87	0.164	2.475	0.4964	0.264	0.2590	0.025	0.064
Stems								
1	4.53	0.119	1.504	0.4242	0.116	0.1828	0.020	0.020
2	3.18	0.158	0.437	0.3593	0.191	0.2710	0.129	0.016
3	2.98	0.102	0.335	0.4227	0.149	0.2547	0.143	0.063
4	3.77	0.134	0.292	0.5262	0.167	0.2391	0.237	0.254
5	4.22	0.153	0.278	0.5572	0.168	0.2941	*	*
6	6.21	0.261	1.934	0.5215	0.131	0.4119	0.015	0.025
7	5.99	0.057	2.206	0.5050	0.106	0.3376	0.019	0.019
Dead leaves								
1	8.23	0.102	1.270	0.6335	0.632	0.6115	0.025	0.047
2	8.78	0.111	0.213	0.7048	1.395	0.4881	0.205	0.059
3	7.58	0.123	0.160	0.9481	0.179	0.6236	0.041	0.031
4	7.81	0.140	0.205	0.9565	0.287	0.7248	*	0.041
5	8.53	0.117	0.132	0.1429	0.112	0.5588	0.027	0.027
6	8.54	0.145	1.262	0.8530	0.527	0.6050	*	*
7	8.61	0.175	1.154	0.8725	0.426	0.5630	*	*
Roots								
1	12.14	1.904	0.901	0.7817	0.991	0.2135	0.082	0.233
2	14.88	2.975	0.353	0.8123	1.254	0.2851	0.296	0.273
3	15.90	3.567	0.183	0.8808	1.018	0.1595	0.376	0.625
4	23.67	7.075	0.161	0.9972	0.911	0.0781	0.301	1.210
5	21.21	5.854	0.183	0.9816	0.945	0.1581	0.017	0.686
6	15.34	2.855	1.303	0.8881	0.938	0.4478	0.041	0.330
7	23.82	6.997	1.454	0.8579	0.622	0.3002	0.058	0.299

* Omitted because of insufficient material.

iron in all the plants was greater in the roots than in the stems and the blades. The percentages of iron in the roots of series 4 and 5 were greater than in 6 and 7.

The results of the ash analyses, recalculated to show the total amounts per plant in grams, are presented in tables XVII and XVIII. Table XVII

TABLE XVII

AVERAGE TOTAL AMOUNT OF ASH CONSTITUENTS IN PLANTS HARVESTED NOVEMBER 20, EXPRESSED IN GRAMS; RECALCULATED FROM TABLE XV

SERIES	AVERAGE TOTAL DRY WEIGHT (OVEN DRY)	ASH	Si	K	P	Ca	Mg
Blades	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	66.3	3.41	0.044	1.180	0.238	0.185	0.113
2	66.6	2.81	0.041	0.636	0.264	0.274	0.256
3	36.3	1.69	0.032	0.196	0.242	0.182	0.196
Roots (air dry)							
1	23.7	5.915	1.898	0.421	0.142	0.126	0.073
2	28.7	5.000	1.307	0.213	0.129	0.219	0.135
3	11.2	3.199	1.052	0.043	0.084	0.058	0.031
4	5.8	1.840	0.662	0.017	0.042	0.032	0.014
5	6.0	2.029	0.659	0.020	0.051	0.037	0.016

shows that the blades of series 1 and 2 were nearly the same in dry weight per plant; that series 1 had a higher total ash content than series 2 chiefly because of the higher amount of potassium in series 1 than in 2; that silicon was very nearly equal in the two series; and that phosphorus, calcium, and magnesium were all present in greater amounts in series 2 than in series 1. Even series 3, the blades of which had half the dry weight of series 1, contained more phosphorus and magnesium and nearly the same amount of calcium as series 1. The roots of series 2 contained more calcium and magnesium than those of series 1, but the roots of series 3 were lower in all the ash constituents studied.

Table XVIII shows that at the age of seven and one-half months the total amounts of ash constituents in the plants depended more upon the size of the plant than upon the percentage of potassium. Both the stems and roots of series 2 weighed less than those of series 1, but the stems of series 2 had more silicon, calcium, magnesium, and sodium than series 1; and the roots of series 2 had more ash, silicon, phosphorus, calcium, magnesium, sodium, and iron than in series 1. It was therefore by no means general that the plants deficient in potassium contained greater total amounts of ash constituents per plant than the controls, probably because the former

TABLE XVIII

AVERAGE TOTAL AMOUNTS OF ASH CONSTITUENTS IN PLANTS HARVESTED APRIL 27, EXPRESSED IN GRAMS; RECALCULATED FROM TABLE XVI

SERIES	AVERAGE TOTAL DRY WEIGHT	ASH	SI	K	P	CA	MG	NA	FE
Blades (oven dry)	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	199.8	14.7	0.235	4.345	1.019	1.090	0.859	0.028	0.044
2	263.0	20.7	0.247	4.737	1.405	2.214	1.478	0.097	0.045
3	68.6	3.9	0.071	0.497	0.448	0.191	0.354	0.021	0.037
4	14.4	0.9	0.019	0.051	0.115	0.031	0.092	0.005	0.004
5	14.9	1.0	0.026	0.074	0.118	0.036	0.081	0.004	0.008
6	124.8	8.1	0.188	2.203	0.658	0.348	0.339	0.031	0.039
7	105.8	7.3	0.017	2.618	0.525	0.279	0.274	0.026	0.067
Stems (oven dry)									
1	299.4	13.47	0.357	4.50	1.27	0.347	0.547	0.059	0.059
2	268.9	8.55	0.425	1.17	0.97	0.513	0.729	0.347	0.043
3	35.9	1.07	0.037	0.12	0.15	0.053	0.091	0.051	0.023
4	4.9	0.18	0.006	0.01	0.03	0.008	0.012	0.012	0.012
5	6.5	0.27	0.009	0.02	0.04	0.010	0.019	.....	.....
Roots (air dry)									
1	108.5	13.17	2.06	0.977	0.848	1.075	0.232	0.089	0.253
2	106.8	15.89	3.18	0.377	0.867	1.339	0.304	0.316	0.291
3	27.8	4.42	0.99	0.051	0.245	0.283	0.044	0.104	0.174
4	8.2	1.94	0.58	0.013	0.082	0.075	0.006	0.025	0.099
5	11.2	2.40	0.66	0.021	0.111	0.106	0.018	0.002	0.077
6	47.6	7.30	1.36	0.620	0.423	0.446	0.213	0.019	0.157
7	40.5	9.52	2.79	0.589	0.347	0.252	0.121	0.023	0.121

were generally smaller. However, the fact that they did contain more in the places just mentioned increases the importance of the differences in percentage and leads to the conclusion that the absorption of potassium tends to decrease the absorption of such elements as phosphorus, calcium, magnesium, and iron.

Discussion

The economic importance of physiological studies of potassium deficiency in sugar cane lies in the effects upon the growth, resistance to diseases, and quality of the juices exerted by potassium. A survey of the literature dealing with sugar cane shows that there is no real agreement regarding the value of potash fertilization, inasmuch as the results of some experiments indicate a response while others do not. No attempt will be made to cite all the pertinent references, but a few representative ones will be mentioned as illustrations. The importance of potassium in increasing growth and yield of cane has been discussed by STEWART and VERRET (**68**) and by MOIR (**53**), who stress its necessity in the early growth of cane.

NAQUIN (54) has found that there is a greater response to potash fertilization in hollows than on knolls or in thin soil. AYRES (8) found that cane in some crops used well over 800 lb. K_2O per acre, illustrating the tremendous demand made by cane for potash. MCGEORGE (50), on the other hand, states that the majority of Hawaiian soils do not respond to potash fertilization.

Among those reporting gains in juice quality resulting from the application of potassium, the following may be mentioned: AGEE (1), ALEXANDER (3, 4), HARRISON (27), and PRATT (61). FERRIS (19) found no increase in yield due to the addition of potassium. VERRET (71) reported that there was no effect whatever on the quality ratio from the use or omission of phosphoric acid or potash or both. According to GEERLIGS (21, 22), generally a juice of low purity is highest in potassium, while juices rich in sucrose contain less potassium, and those varieties which make the most sugar generally have the least potassium. GEERLIGS concluded that many other factors have a greater effect upon quality than potash. That this condition of non-conformity of results of potash studies still exists is shown by a recent report by AGEE (2), in which the results of several experiments are summarized. When one considers the sources of error in field experiments as at present conducted, there is no wonder that the results are not always in agreement. Of course the response from applications of potash depends upon the amount of potassium already in the soil and many other factors. MOIR (53) stresses the balance as important, stating that the application of plant foods aids crop quality when the element lacking is supplied, and continues to do so until some other element becomes limiting or until one is added in too large amount.

The importance of potassium in increasing resistance to disease in plants is well known. Sugar cane is no exception. LEE (43) and LEE and MARTIN (44) report that potassium aids in increasing resistance toward eye spot. MOIR (53) suggests that the increase in cutin due to potassium found by HARTT (28) points to the effect of potassium in lessening damage due to cane diseases such as eye spot and brown stripe. He also states that the large cavities in the cortex of the roots and the poor root hairs in the potassium-deficient plants reported by HARTT may suggest one possible cause of the root failure complex or Lahaina disease. The evidence is inconclusive regarding a relationship between a lack of potassium and brown stripe disease. HANCE (26) by spectroscopic analysis found that cane plants susceptible to brown stripe were deficient in barium, potassium, magnesium (or copper), and cadmium (or zinc). SHEPARDSON (64), however, found no apparent correlation between brown stripe and potassium, but suggested further studies concerning silicon and that disease. In gen-

eral there is need for more investigation of the relation between potassium and disease resistance in sugar cane.

The preceding brief review illustrates the need for more fundamental physiological studies of potassium in sugar cane.

Although similar in plan and purpose, the present studies are not absolutely comparable with those carried on in Chicago (**28**), for several reasons. In the Chicago experiment the variety used was Louisiana Purple (Cheribon), while H 109 was used in the Honolulu studies. The growing conditions also differed considerably. In the former investigation the plants were grown during the winter and spring in Chicago and during much of the time the light conditions were poor, although supplemented by electricity. In Honolulu the length of day and amount of light were more nearly uniform and better adapted for the growth of cane. The temperature also differed, the Chicago greenhouse being kept around 90° F., whereas in Honolulu the temperature at times reached 110° F. Because of the differences in light and temperature, the growth rate of the plants in the present investigation was considerably greater than that of the first study. The nutrient solutions used in Honolulu were less concentrated than those used in Chicago but were renewed more frequently. Also silicon, manganese, and titanium were used in these studies and not in the former. Iron was added as ferric phosphate in the former, whereas ferrous sulphate was used in the latter investigation. Undoubtedly there were other differences in the growing conditions of the two experiments. Secondary growth was purposely discouraged in the Chicago experiment, whereas in Honolulu the secondary shoots were allowed to develop. The writer thus agrees with the criticism of VAN DEN HONERT (**70**) that the plants in Chicago were grown under unfavorable light conditions. Notwithstanding these differences in variety, light, temperature, growth rate, and nutrient solutions, similar symptoms of potassium deficiency occurred in both and it is felt that broad comparisons are justified between the two investigations.

Evidence of the difference in the growth rates of the plants grown in Chicago and Honolulu is given in table XIX. This shows that in one month the main stalks of the plants grown in Honolulu were nearly as tall as those in Chicago in about five months, when the lengths of the longest leaves are compared. The first symptoms of potassium starvation developed in Honolulu within one month after watering with the nutrient solutions was begun, while four months elapsed in Chicago before deficiency symptoms appeared. While other factors no doubt contributed to these differences in growth and development of symptoms, yet it is felt that one of the most important causes was the amount of light. In this connection mention should be made of an unpublished study conducted by Miss MARJORIE

BODWELL in 1931, at that time a student with the writer at Connecticut College. Miss BODWELL grew buckwheat and soy bean plants with and without potassium under several intensities of light. The plants in the most intense light were the first to exhibit symptoms of potassium starvation, the others following in the order of decreasing intensities of light.

TABLE XIX

COMPARISON OF GROWTH: CHICAGO AND HONOLULU

SOLUTION NO.	CHICAGO STUDIES			HONOLULU STUDIES			
	K	LENGTH OF LONGEST LEAF				K	SOLUTION NO.
		1 MO. 15 DAYS	5½ MO.	1 MO. 5 DAYS	3½ MO.		
	p.p.m.	*cm.*	*cm.*	*cm.*	*cm.*	*p.p.m.*	
1	703.8	88.9	170.2	140.7	198.6	87.9	1
4	39.0	67.3	143.5	147.1	198.2	39.0	2
3	3.9	68.9	151.1	133.7	160.5	3.9	3
2	0.0	71.7	139.7	125.8	135.9	0.0 (+Na)	4
				120.3	136.9	0.0 (–Na)	5

The effect of light upon the absorption and utilization of potassium by plants has been studied by several workers. JOHNSTON and HOAGLAND (**39**) have given evidence that plants absorb more potassium during the day than at night and suggested that the energy of light may be an important factor governing the absorption of nutrient ions by plants. NEMEC (**56**) grew rye under colored glass bell jars and found that plants under green jars absorbed less potassium than those in full sunlight, while those under violet and red jars contained more potassium than those in sunlight. NIGHTINGALE (**57**) refers to STOKLASA who found that sugar beet seedlings in darkness when supplied with potassium and sugar accumulated much more dry matter than plants in the dark lacking potassium but supplied with sugar; while in the light, the amount of sugar absorption was only a little higher in the plants supplied with potassium than in those lacking it. It was therefore suggested that potassium to some extent assumes the rôle of light. A connection between light and potassium seems substantiated. Since in the present studies equal amounts of potassium were absorbed by sugar cane plants during twelve hours of darkness and twelve hours of daylight (table XII), it would seem that light must affect the utilization of potassium by sugar cane rather than its absorption. Whether this may be due to the photoelectric properties of potassium or is merely a matter of limiting factors will be discussed in the second paper of this series.

Absorption of potassium

The amount of potassium absorbed probably depends more upon its amount and availability in the soil than upon the rate of its utilization within the plant, since considerable evidence has been given by Bartholomew and Janssen (9) indicating that plants may indulge in a luxury consumption of potash. Undoubtedly the age of the plant is also an important factor influencing the absorption of potassium, particularly when young. Evidence of this is given in table XI, showing that one month after watering with the nutrient solutions was begun, the plants of series 1 and 2 were absorbing potassium at the same rate. The plants were about the same size at that time, no symptoms of potassium deficiency having yet appeared in series 2. As is shown in figure 15, early in November, the plants of series 1 definitely surpassed those of series 2 in growth. It is interesting that on November 13 and 14, series 1 showed the ability of absorbing double the amount of potassium absorbed by series 2, according to table XII. It would seem that when supplied with 39–88 p.p.m. potassium, the amount absorbed during the first month is limited by the size of the plant, probably in particular by the size of the root system; but when there is 3.9 p.p.m. potassium or less, the amount of potassium is the limiting factor, and potash starvation symptoms develop earlier. Inasmuch as the absorption of potassium seems to be limited by the size of the young plant, it is apparent that large applications of potash in the field during the first month may not be economical.

André and Demoussy (6, 7) have stressed the importance of the mobility of potassium. The absorption of considerable amounts of potassium may decrease the amounts of certain other elements absorbed, which may offer a partial explanation for the depressing effect of potassium upon the growth and juice quality of cane which has been mentioned by Agee (2). In the present study, little evidence of a depressing effect upon growth was obtained. Three weeks after starting the plants in the nutrient solutions, the plants of series 2 slightly surpassed those of series 1 in growth, as shown in table II. This difference held also in nine weeks for certain measurements (tables III–V), but at that time the average height of the highest dewlap of the plants of series 1 was higher than that of series 2; and from then to the end of the experiment the growth of the plants of series 1 steadily surpassed that of series 2, as shown in figure 15.

Sugar cane plants deficient in potassium were found to absorb more of certain other elements on the percentage basis than did the controls, a condition similar to that studied in other plants by various investigators: Anderson *et al.* (5), Davis (16), Fonder (20), Ginsburg (24), Hoagland (32), Owen (59), and others. Two months after starting the plants in the nutrient solutions, the potassium-deficient plants were absorbing more

silicon, phosphorus, calcium, and magnesium than the controls, as shown by table XV. That this was a real difference in amount is shown by the fact that the plants of series 4 and 5 had the largest percentage of total ash, particularly in the roots, and is also shown by the greater total amounts of ash constituents in the plants deficient in potassium than in the controls, found in some of the series, as shown in tables XVII and XVIII. This difference in absorption did not continue to the end of the experiment, as at the time of final harvest the potassium-deficient plants had the lowest percentage of total ash in the tops, although they still had the highest percentage in the roots. The absorption of phosphorus continued to be greater in the plants starved for potassium to the end of the experiment, which may have an important bearing upon the activity of amylase, as will be mentioned in the second paper in this series. The final percentages of calcium and magnesium varied irregularly in the different parts of the plants, except that calcium was highest in the blades of the plants of series 1 and 2.

Determinations were also made of the amounts of iron and sodium in the plants harvested in April. The percentages of iron in the blades, stems, and roots of the plants deficient in potash were found to be higher than in the controls, showing that a deficiency in potassium leads to the absorption of a greater amount of iron. Sodium was lower in amount than potassium in the blades and the stems of all the plants, even those supplied with more sodium than potassium. These comparisons are taken from table XVI.

The addition of potassium to the potassium-deficient plants resulted in a rapid absorption of that element and a considerable increase in growth. The steps in recovery outlined in the results were similar to those obtained in the field, according to MOIR (personal communication). Table XVI shows that in some instances the plants of series 6 and 7 contained higher percentages of potassium than did the controls. To a certain extent this may be due to the fact that the absorption of potassium was more rapid than the resulting growth. The plants of series 7 contained more potassium than did those of series 6 in all organs except the dead leaves. This higher percentage in series 7 led to a considerably greater growth, series 7 surpassing series 6 to a marked degree during the latter part of the experiment. The addition of potassium to the plants of series 6 and 7 also decreased the percentages of phosphorus and magnesium in those plants.

The effect of the addition of potassium upon the distribution of iron within the plant is important. Table XVI shows that the plants of series 6 and 7 had considerably lower percentages of iron in the roots than those of series 4 and 5, indicating that the addition of potassium to the former beginning in January had the important effect of decreasing the percentage of iron in the roots as a part of their recovery from potassium deficiency. Table XVI also shows that the stems of the plants deficient in potassium

contained larger percentages of iron than the controls, whereas the plants of series 6 and 7 contained nearly the same amount in the stems as the controls. It is evident that the addition of potassium to the potassium-deficient plants resulted in a decrease in the percentage of iron within the roots and stems. That this was not entirely the effect of a greater distribution of the iron within the larger stems is shown by the relative amounts of iron in the blades and stems of the plants of series 6 and 7. Table XVI shows that the percentages of iron in the blades of series 6 and 7 were nearly double or treble the percentages in the stems of those plants, whereas in series 1–4 the percentages of iron in the blades were equal to or less than the percentages in the stems. It seems that the addition of potassium to the plants starved for that element caused some of the iron which had accumulated in the stems to pass up into the blades. This may be explained by assuming that the addition of potassium increased the turgidity of the leaves and thus increased the speed of the upward movement of the transpiration stream and the mass movement of the iron. That potassium affects turgidity is well known, having been established by COPELAND (13) as early as 1897. Evidence that the rate of transpiration is affected by the supply of potassium was obtained in a test using the cobalt chloride paper method, in which equal shades of pink were developed in 55 seconds on a blade of one of the plants of series 1, and in four minutes on a blade of one of the plants of series 3, these blades having similar exposures.

To summarize, several generalizations may be offered regarding the absorption of potassium by the sugar cane plant, from the studies reported in this paper:

1. Since equal amounts of potassium were absorbed by day and by night, the absorption of potassium does not seem to be affected by light.

2. With the exception of the first three or four weeks, the amount of potassium absorbed is directly proportional to the amount supplied, within the limits of this experiment.

3. During the first month the absorption of potassium is limited by the size of the plant.

4. Cane plants deficient in potassium absorb more phosphorus and iron, at least during the first seven months of their growth. They also absorb more calcium, magnesium, and silicon during the first two or three months.

5. The addition of potassium to plants deficient in that element results in a rapid absorption of potassium, a decrease in the intake of phosphorus and magnesium, and causes some of the iron which had accumulated in the stems to pass up into the blades.

MIGRATION OF POTASSIUM

There was a greater percentage of potassium in the blades than in the stems, and the stems in turn were higher in potassium than were the dead

leaves (table XVI). In every series the percentage of potassium in the dead leaves collected in April was not only lower than in the blades harvested at the same time but also lower than in the blades of the November material. Therefore the potassium migrated from the dying leaves to the living top of the sugar cane in this experiment, as has also been shown by BONAME (**10**). BARTHOLOMEW and JANSSEN (**9**), JAMES (**36**), JANSSEN and BARTHOLOMEW (**37**), NIGHTINGALE and coworkers (**57**), and others have mentioned this in connection with other plants. This migration is against the diffusion gradient.

If the percentage of potassium in the dead leaves is subtracted from the percentage in the blades (table XVI), a rough estimate of the amount of migrated potassium is obtained. Then if the ratio is taken of the percentage of migrated potassium to the percentage of potassium left in the dead leaves, the results in table XX are obtained. This shows that the relative amounts of migration are in this order: $2 > 3 > 5 > 7 > 4 > 1 > 6$. If this is compared in a similar manner with the percentages of the following in blades: total ash, K, Si, P, Ca, Mg, Fe, Na, and H_2O, it is found that there is no relation between any of the ash constituents studied and the migration of potassium. The moisture content decreased in this order: $1 > 6 > 7 > 4$ and $5 > 3 > 2$. Omitting the plants of series 6 and 7, it is found that the migration of potassium occurred in this order $2 > 3 > 5 > 4 > 1$. The water content decreased in the order: $1 > 4$ and $5 > 3 > 2$. Possibly the plants of series 6 and 7 should be considered separately inasmuch as their treatment was changed during the course of the experiment. Of the two, series 6 had the greater moisture content and the lower migration of potassium while series 7 had the greater migration of potassium and the lower percentage of water. The conclusion seems inevitable that the greatest migration of potassium occurred in the blades having the lowest water percentage, and that there was no relationship between the amount of migration and the percentage

TABLE XX

RELATIVE AMOUNTS OF MIGRATION OF POTASSIUM

SERIES	% POTASSIUM MIGRATED — % POTASSIUM LEFT IN DEAD LEAVES
1	0.71
2	7.4
3	3.5
4	0.73
5	2.0
6	0.4
7	1.1

of potassium in the plant. In other words, migration did not occur most readily in those plants which needed it most; therefore the translocation of potassium is not an adaptation in potassium-deficient plants, but occurs there more than elsewhere only when those plants at the same time have the lowest moisture content. The suggestion made by PENSTON (**60**) is pertinent, that migration is possibly related to age rather than to a deficiency in potassium. It is probably an exception in the present study that the plants of series 2 contained less water than those of series 3, 4, or 5, possibly explained by their greater size and the fact that for some undetermined reason it was practically impossible to keep the plants of series 2 adequately supplied with water, as they required even more than series 1. It is probably more generally true that the moisture percentage is directly proportional to the amount of potassium supplied, since several investigators (**11, 28, 35, 38, 72**) have reported lower moisture contents or higher percentages of dry matter in potassium-deficient plants; hence the migration of potassium may in general occur most in the plants which benefit most by that condition. It is felt that statements in the literature regarding such translocations often verge on the teleological, when it is implied or stated that plants deficient in potassium can transfer the little potassium they contain from a place where it is no longer in use to where it is needed. The results herein reported show that this is not an adaptation in potassium-deficient plants, since it occurred most readily in the plants of series 2, which contained considerably more potassium than the plants of series 3, 4, or 5, and occurred equally well for series 1 and 4. DOWDING (**18**) also found that a translocation of potassium occurs within the embryonic cone during bud elongation in the spruce, and those plants were not deficient in potash.

Probably several factors are involved in the migration of potassium. In leaves it may simply be a balance of forces between the transpiration of the plant as a whole and that of the lower leaf. When the lower leaf contains very little water the pull of transpiration from above readily overcomes the pull of the individual dying leaf, but when the lower leaf contains a considerable amount of water, such is not the case. The failure of the development of the abscission layer in plants in extreme potassium deficiency, found in cane by NAQUIN (**55**), who stated that in potassium deficiency the dead leaves adhere to the stalk, and reported in the tomato by LIPMAN (**46**), is another important factor. Without the usual abscission layer the leaf would tend to remain longer on the plant, thus allowing migration. KOSTYTSCHEW and ELIASBERG (**42**) were among the first to find that potassium can be extracted from plant tissues *in toto* by water. Since JANSSEN and BARTHOLOMEW (**37**) and others have also found that most of the potassium in plants is soluble in water, it seems that potassium is

present in a readily movable form. Undoubtedly other factors are involved, because the situation is not always so simple. Potassium not only migrates into the upper leaves, but also into fruits and to the meristematic tips of roots as reported by LIPMAN (**46**). If electric currents of mitotic origin similar to those found in wheat plants by COLLA (**12**) are of general occurrence, possibly these might aid in explaining the migrations of potassium to meristematic regions. The suggestion proposed by MCGEORGE (**52**) that bases may be absorbed by lignin and displaced by other bases or by hydrogen, and that this interchange may play a part in ionic movement through plant tissue, may contribute to the explanation of the mechanism of the translocation of potassium. In short, the forces involved in the migration of potassium from the older regions of plants to young developing portions possibly include transpiration, base exchange, and electrokinetic phenomena.

Of the ash constituents studied, potassium was the only one which exhibited the ability to migrate. Table XVI shows that the dead leaves of all the plants had a higher percentage of total ash than had the green blades. The silicon, phosphorus, calcium, magnesium, iron, and sodium varied irregularly in most of the groups, being greater in amount in the dead leaves than in the blades, with occasional exceptions. This is to be expected, since with the exception of sodium the elements mentioned are known to enter into organic compounds, either in wall material or protoplasm; potassium exists for the most part in simple form. Another factor involved is the greater mobility of potassium as compared with the other elements, which has been stressed by ANDRÉ and DEMOUSSY.

IRON TOXICITY

An interesting controversy has occurred in Java as to whether certain symptoms in sugar cane may be attributed to potassium deficiency or to iron toxicity. In the condition known as Kalimati disease (so called from the name of the principal plantation where it occurs), the symptoms are very similar to those of potassium deficiency and the addition of potassium has been found to overcome the trouble. WILBRINK (**73**) thinks that Kalimati disease is caused by potassium deficiency. KONINGSBERGER (**40, 41**), however, is of the opinion that the disease is due to iron toxicity, which occurs particularly with ferrous iron when ammonium nitrogen is used instead of nitrate nitrogen. He thinks that the disease is overcome by the addition of potassium either because potassium counteracts the injurious action of iron or because it may stimulate nitrification. That the Kalimati disease is overcome by the addition of potassium is insufficient ground for calling the disease potassium deficiency, according to KONINGSBERGER, who feels that that is no better than to speak of "quinine deficiency" in the case of malaria.

Whether one agrees with WILBRINK or with KONINGSBERGER, it is interesting that a toxic effect of iron, which can be overcome by the addition of potassium, occurs in both Kalimati disease and potassium deficiency. HOFFER (34) reported that the addition of potassium to corn plants having accumulations of iron at the nodes cleared up that condition but the accumulation of aluminum was not affected. HOFFER found the vessels of plants deficient in potassium clogged with iron, and these vessels became clear upon the addition of potassium. He pointed out that this accumulation causes the breakdown, either by precipitation or by coagulation, of the cell contents of certain joint tissues, interfering with translocation. Furthermore, HARVEY (30) stated that in maize grown in acid soil there may be a deposit of Fe_2O_3 in the vascular tract sufficient to interfere with translocation, and that firing of the leaves may be observed under such conditions. MCGEORGE (51) found that both potassium and phosphorus aid in the distribution of iron and that any growth-retarding factor may induce accumulations of iron and aluminum in the nodes of cane. In the present study iron was found to be higher in the potassium-deficient plants than in the controls, particularly in the roots and stems. Iron occurred especially at the nodes of the plants deficient in potassium but not of the controls.

Either because of this nodal accumulation of iron or on account of an upset in the synthesis of proteins resulting in abnormal protoplasm in the sieve tubes and companion cells, or for both reasons, a necrosis of the phloem occurred in the plants deficient in potassium. The derangement in the formation of proteins will be discussed fully in the second paper of this series. In the previous study (28) a little evidence was obtained of derangements of the phloem in the plants deficient in potassium. Reference to the section dealing with microchemical results will show that further evidence of phloem necrosis in the plants deprived of potash has been offered in the present study. The evidence consisted of a dark discoloration of the bundles of the stems and of both sieve tubes and companion cells of the bundles of the midribs. These discolorations were noted as early as the latter part of October. Furthermore, it has recently been found by MARTIN (49) that similar discolorations of the bundles in cane stems occur throughout the Hawaiian Islands, and there is some evidence that they are related to potash deficiency. This condition has been termed "internal stalk necrosis."

These accumulations occur more at the nodes than at the internodes, possibly because of the anastomosis of the bundles in the former location. Perhaps another factor involved in the nodal accumulations of iron is the tannin content which, according to HALDEN (25), is particularly large in the nodes. It would seem that the insolubility of iron tannate must play a

9. BARTHOLOMEW, R. P., and JANSSEN, G. The rate of absorption of potassium by plants and its possible effect upon the amount of potassium remaining in soils from applications of potassium fertilizers. Arkansas Sta. Bull. 265. 1931.
10. BONAME, PH. Culture de la canne à sucre à la Guadeloupe. Paris. 1888.
11. BURRELL, R. C. Effect of certain deficiencies on nitrogen metabolism of plants. Bot. Gaz. **82**: 320–328. 1926.
12. COLLA, S. Sui fenomeni bioelettrici in rapporto con l'accrescimento della piante. Nuov. Gior. Bot. Ital. **34**: 1281–1295. 1928.
13. COPELAND, E. B. The relation of nutrient salts to turgor. Bot. Gaz. **24**: 399–416. 1897.
14. CRUTCHFIELD, C. L. X-ray photography of mineral accumulations in plants. Plant Physiol. **4**: 145–154. 1929.
15. DAVIS, L. E. Manganese as an essential element in the growth of sugar cane. Hawaiian Pl. Record **35**: 393–400. 1931.
16. DAVIS, M. B. Investigations on the nutrition of fruit trees: Some effects of deficiencies of nitrogen, potassium, calcium, and magnesium, with special reference to the behaviour of certain varieties of apple trees. Jour. Pomol. & Hort. Sci. **8**: 316–344. 1930.
17. DOMONTOVICH, M. K., and ZHELEZNOV, P. A. Feeding of plants via the leaves with salts of potassium and of magnesium. (Transl. title.) Nauch. Agron. Zhur. (Jour. Landw. Wiss.) **7**: 134–143. 1930.
18. DOWDING, E. SILVER. The regional and seasonal distribution of potassium in plant tissues. Ann. Bot. **39**: 459–474. 1925.
19. FERRIS, E. B. Sugar cane for syrup making. Mississippi Agr. Exp. Sta. Bull. 199. 1921.
20. FONDER, J. F. Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. Jour. Amer. Soc. Agron. **21**: 732–750. 1929.
21. GEERLIGS, H. C. P. Over den samenhang van het kali- en het suikergehalte van het suikerriet. Archief Suikerind. Nederl.-Ind. (Java) **18**: 108–117. 1910.
22. ————. The correlation between the sugar and potash contents of cane juice. Int. Sug. Jour. **13**: 417–421. 1911.
23. GORTNER, R. A. Outlines of biochemistry. John Wiley & Sons, Inc., New York. 1929.
24. GINSBURG, J. M. Composition and appearance of soybean plants grown in culture solutions each lacking a different essential element. Soil Sci. **20**: 1–13. 1925.

25. HALDEN, G. H. Dark color of cane juice. Hawaiian Pl. Record **17**: 10. 1917.
26. HANCE, F. E. Unpublished data in letter to the Director, Exp. Sta., H. S. P. A. March, 1930.
27. HARRISON, W. H. Report of the Imperial Agr. Chem., Sci. Rpt. Agr. Res. Inst. Pusa. **1927–28**: 30–39. 1928.
28. HARTT, CONSTANCE E. Potassium deficiency in sugar cane. Bot. Gaz. **88**: 229–261. 1929.
29. HARTWELL, B. L., and DAMON, S. C. The value of sodium when potassium is insufficient. Rhode Island Agr. Exp. Sta. Bull. 177. 1919.
30. HARVEY, R. B. Plant physiological chemistry. Century Co., New York. 1930.
31. HEINRICH, H. Über die Wirkung des Natrons neben dem Kali als Nahrstoff der Pflanzen. Zeitschr. Pflanzenernähr. Düngung & Bodenk. **10A**: 299–323. 1928.
32. HOAGLAND, D. R. The absorption of ions by plants. Soil Sci. **16**: 225–246. 1923.
33. HOFFER, G. N. Testing corn stalks chemically to aid in determining their plant food needs. Indiana Agr. Exp. Sta. Bull. 298. 1926.
34. ————, and TROST, J. F. The accumulation of iron and aluminum compounds in corn plants and its probable relation to root rots. Jour. Amer. Soc. Agron. **15**: 323–331. 1923.
35. JAMES, W. O. Studies of the physiological importance of the mineral elements in plants. I. The relation of potassium to the properties and functions of the leaf. Ann. Bot. **44**: 173–198. 1930.
36. ————. Studies of the physiological importance of the mineral elements in plants. II. Potassium: its distribution, movement, and relation to growth in the potato. Ann. Bot. **45**: 425–442. 1931.
37. JANSSEN, G., and BARTHOLOMEW, R. P. Rôle of potassium. Arkansas Sta. Bull. 246. 27–29. 1929.
38. ————, and ————. The influence of the potash concentration in the culture medium on the production of carbohydrates in plants. Jour. Agr. Res. **40**: 243–261. 1930.
39. JOHNSTON, E. S., and HOAGLAND, D. R. Minimum potassium level required by tomato plants grown in water cultures. Soil Sci. **27**: 89–109. 1929.
40. KONINGSBERGER, V. J., and VAN DEN HONERT, T. H. Over de Oorzaak der z. g. "Kalimati-ziekte." Archief Suikerind. Nederl.-Ind. (Java) **39**: Deel I: 28–39. 1931.

41. ————, and ————. Nogmaals over de oorzaak der z. g. Kalimati-ziekte. Antwoord op de kritiek van Mej. Dr. G. Wilbrink. Archief Suikerind. Nederl.-Ind. (Java) **39**: Deel I: 161–166. 1931.

42. KOSTYTSCHEW, S., and ELIASBERG, P. Über die Form der Kaliumverbindungen in lebenden Pflanzengeweben. Zeitschr. physiol. Chem. **111**: 228–235. 1920.

43. LEE, H. A. The effect of potash fertilizers on eye spot at the Waimanalo Sugar Company. Hawaiian Pl. Record **31**: 284–287. 1927.

44. ————, and MARTIN, J. P. Effect of fertilizer constituents on the eye-spot disease of sugar cane. Ind. & Eng. Chem. **20**: 220–224. 1928.

45. ————, and MCHARGUE, J. S. The effect of a manganese deficiency on the sugar cane plant and its relationship to Pahala blight of sugar cane. Phytopath. **18**: 775–786. 1928.

46. LIPMAN, J. G. Report of the director, 50th Ann. Rpt. New Jersey Agr. Exp. Sta. 1929 (p. 36: Horticultural biochemistry).

47. MARTIN, J. P. Potash studies in relation to sugar cane. Director's Monthly Rpt. Exp. Sta., H. S. P. A. November, 1931.

48. ————. Pahala blight. Rpt. Exp. Sta. Com., H. S. P. A. 1931. p. 56.

49. ————. Internal stalk necrosis. Director's Monthly Rpt., Exp. Sta., H. S. P. A. August 10, 1932.

50. MCGEORGE, W. T. The availability of potash in Hawaiian soils. H. S. P. A. Exp. Sta. Bull. Agr. and Chem. Series 48. 1924.

51. ————. The influence of aluminum, manganese and iron salts upon the growth of sugar cane, and their relation to the infertility of acid island soils. H. S. P. A. Exp. Sta. Bull. Agr. and Chem. Series 49. 1925.

52. ————. Chemical equivalent base exchange reactions in plants. Plant Physiol. **7**: 119–124. 1932.

53. MOIR, W. W. G. The plant food problem. Rpt. Assn. Hawaiian Sug. Tech. 9th Ann. Meeting. pp. 175–203. 1930.

54. NAQUIN, E. E. Benefits derived from field experiments on unirrigated plantations. Proc. Assn. Hawaiian Sug. Tech. 10th Ann. Meeting. pp. 71–83. 1931.

55. NAQUIN, W. P. Plant food deficiency and its relation to root rot. Ref. Bk. Sugar Ind. of World **4**: 59–63. 1926.

56. NEMEC, A., and GRAČANIN, M. Influence de la lumière sur l'absorption de l'acide phosphorique et du potassium par les plantes. Compt. Rend. Acad. Sci. (Paris) **182**: 806–808. 1926.

57. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.

58. NOBBE, F., SCHROEDER, J., and ERDMANN, R. Über die organische Leitung des Kalium in der Pflanze. Landw. Vers.-Sta. **13**: 321–399; 401–423. 1871.

59. OWEN, O. The analysis of tomato plants. II. The effect of manurial treatment on the composition of tomato foliage. Jour. Agr. Sci. **21**: 442–451. 1931.

60. PENSTON, NORAH L. Studies of the physiological importance of the mineral elements in plants. III. A study by microchemical methods of the distribution of potassium in the potato plant. Ann. Bot. **45**: 673–692. 1931.

61. PRATT, J. H. Report of the committee on phosphoric acid and potash determinations in cane juices. Hawaiian Pl. Rec. **28**: 34–47. 1924.

62. REED, H. S., and HAAS, A. R. C. Studies on the effects of sodium, potassium, and calcium on young orange trees. California Agr. Exp. Sta. Tech. Paper 11. 1923.

63. SHAW, H. R. Studies on the response of cane growth to moisture. Rpt. Assn. Hawaiian Sug. Tech. 9th Ann. Meeting. pp. 127–147. 1930.

64. SHEPARDSON, H. M. Unpublished data in letter to the Director, Exp. Sta., H. S. P. A. April, 1930.

65. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.

66. SMITH, A. R., and VILBRANDT, F. C. Substitution of sodium compounds for potassium compounds. Jour. Elisha Mitchell Sci. Soc. **42**: 118–121. 1926.

67. STEUERWALD, L. G. L. Over de incrusteerende kleurstof van het suikerriet. Archief Suikerind. Nederl.-Ind. **19**: Deel II: 1543–1557. 1911. (from GEERLIGS, H. C. P. Cane sugar and its manufacture. 2d Rev. ed. Norman Rodger, London. 1924.)

68. STEWART, G. R., and VERRET, J. A. Cane growth studies at Waipio Substation. Rpts. Assn. Haw. Sugar Tech. 8th Ann. Meeting. pp. 221–255. 1929.

69. VAN DEVENTER, W. De Cultuur van het suikerriet op Java. 2de druk. J. H. DE BUSSY, Amsterdam. 1927. (Unpublished transl. by E. D. ROBERTS.)

70. VAN DEN HONERT, T. H. Onderzoekingen over de voedingsphysiologie van het suikerriet. Archief Suikerind. Nederl.-Ind. **40**: Deel III: 1539–1608. 1932.

71. VERRET, J. A. The effect of phosphoric acid and potash on the quality of cane juices. Hawaiian Pl. Record **27**: 117–121. 1923.
72. WALLACE, T. Chemical investigations relating to potassium deficiency of fruit trees. Jour. Pomol. & Hort. Sci. **9**: 111–121. 1931.
73. WILBRINK, G. Kritiek op de voorloopige mededeeling van Dr. V. J. KONINGSBERGER en Dr. T. H. VAN DEN HONERT: Over de oozaak der z. g. Kalimati-ziekte. Archief Suikerind. Nederl.-Ind. **39**: Deel. I: 141–146. 1931.

EXPLANATION OF COLOR PLATE

Color symptoms of potassium deficiency in sugar cane: *A*, typical leaf from control plant; *B*, *C*, and *D*, leaves from plants deficient in potassium. *B* shows the color of a midrib soon after it has become red, while *C* shows the darkening of the discoloration as the leaf ages.

SOME EFFECTS OF POTASSIUM UPON THE AMOUNTS OF PROTEIN AND AMINO FORMS OF NITROGEN, SUGARS, AND ENZYME ACTIVITY OF SUGAR CANE[1]

CONSTANCE ENDICOTT HARTT

(WITH ONE FIGURE)

Introduction

This paper reports the results of determinations of the enzymes invertase, amylase, and ereptase; analyses of total and amino nitrogen, reducing sugars and sucrose; and the hydrogen ion concentrations, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots of plants grown with varying amounts of potassium.

METHODS.—Details of the care of the plants during growth and of the methods employed in harvesting them have been presented in the preceding paper (**26**).

Invertase, amylase, peptase, and ereptase, and total and reducing sugars were determined by the methods used in the studies reported in 1929 (**24**). The titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) was also used for the detection of peptase.

Total nitrogen was determined by the Kjeldahl method, while the Van Slyke method was employed for the estimation of amino nitrogen. The nitrogen determinations were performed by the Chemistry Department of this Station.

Results

1. PHYSICO-CHEMICAL STUDIES OF EXPRESSED SAP

Determinations of the hydrogen ion concentration, titratable acidity, and titration curves were performed using two representative plants of series 1 and 3, harvested December 4, 1931, eleven weeks after starting the plants in the nutrient solutions. The results of the electrometric determinations of the hydrogen ion concentration of the sap expressed from frozen tissues are presented in table I. These data show very little difference between the hydrogen ion concentration of the controls and that of the plants deficient in potassium, the former being slightly more acid than the latter, which is the reverse of the results obtained by REED and HAAS (**40**).

Because of the dark color of the juices, it was necessary to dilute 1 cc. of juice with 20 cc. distilled water before titration. N/5 NaOH was used, with phenolphthalein as indicator. The results are given in table I. This shows that the blades of series 1 were a little less acid than those of series

[1] See footnote 1 of preceding paper.

TABLE I

pH AND TITRATABLE ACIDITY OF EXPRESSED SAP

SERIES	pH	N/5 NaOH TO NEUTRALIZE 1 CC. JUICE
		cc.
Blades		
1	5.26	0.32
3	5.32	0.49
Stems		
1	5.17	0.4
3	5.34	0.3
Roots		
1	5.58	0.09
3	5.64	0.09

3, while in the stems the reverse held, and the roots showed no difference.

By adding varying amounts of sulphuric acid and sodium hydroxide to the expressed sap and determining the hydrogen ion concentration electrometrically, the titration curves were obtained. These are shown in figure 1. A slight change in pH upon addition of acid or alkali indicates that the juice is well buffered, while a greater change means a poorly buffered juice. The graphs show that the blades of series 3 seemed to have a slightly better buffer system than those of series 1; that the buffer systems of the stems were very much alike; but that with roots, the plants of series 1 had a slightly better system on the acid side but those of series 3 were better on the alkaline side. On the whole there was very little difference in the way they reacted to additions of acid and alkali. It would seem, therefore, that differences in hydrogen ion concentration, titratable acidity, and buffer systems were insufficient to explain the results of the enzyme determinations here reported.

2. ANALYTICAL DATA

The results of the nitrogen determinations are presented in tables II and III.

In November, there was a higher percentage of amino nitrogen and lower percentage of protein nitrogen in the plants of series 3 than in the controls, while the total nitrogen remained about the same. These differences were more conspicuous in the blades than in the stems. In April, higher percentages of amino nitrogen, protein nitrogen, and total nitrogen were found in the blades of the plants deficient in potassium, while in the stems the opposite relationship was found. When the total amounts of nitrogen contained in the entire plants are calculated, the results presented in table IV are obtained. These figures show that in November the average dry weight

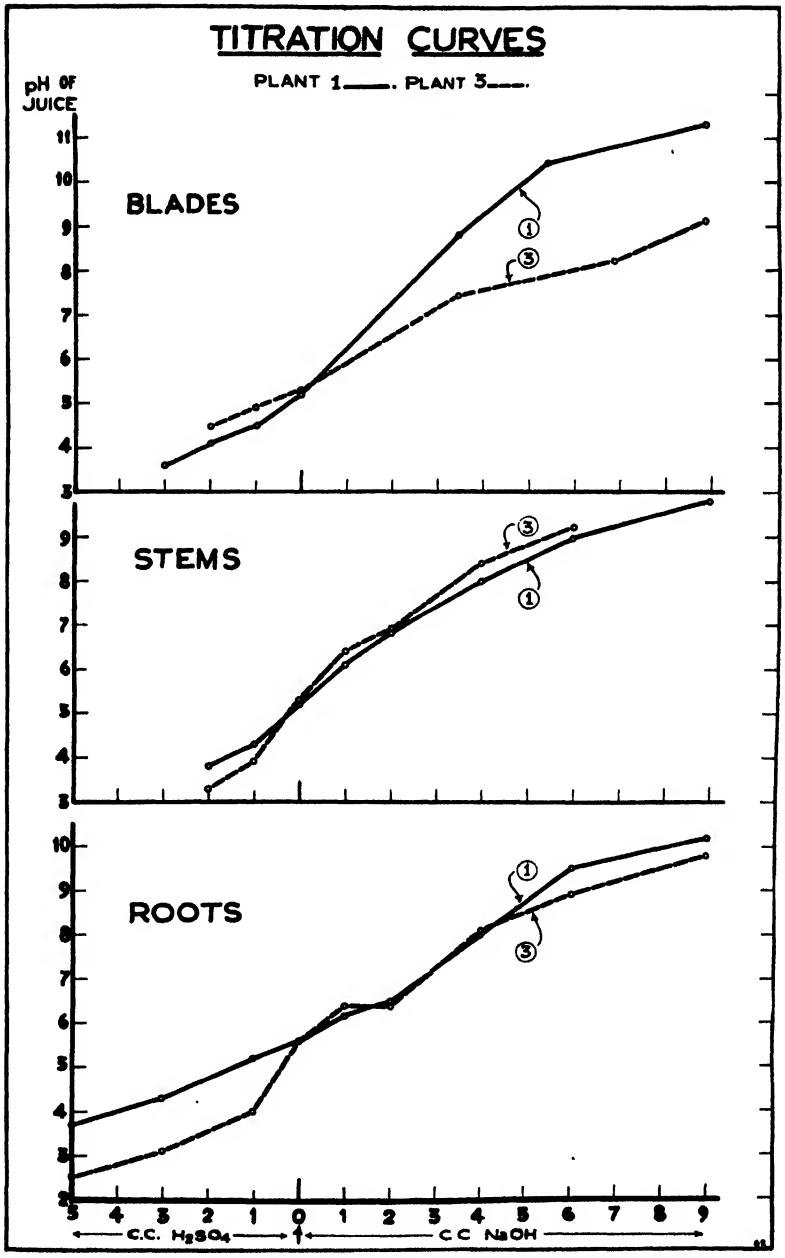


FIG. 1. Effect of addition of varying amounts of acid and alkali upon hydrogen ion concentration of juice expressed from plants 1 and 3.

TABLE II

NITROGEN ANALYSES OF PLANTS HARVESTED NOVEMBER 20, 1931, NINE WEEKS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	NITRATE N	TOTAL N
	%	%	%	%
Blades				
1	0.070 ± 0.011	1.585 ± 0.0004	None	1.655 ± 0.001
2	0.086 ± 0.004	1.605 ± 0.003	None	1.691 ± 0.008
3	0.231 ± 0.001	1.461 ± 0.001	Trace	1.692 ± 0.0004
Stems				
1	0.395 ± 0.006	0.986 ± 0.007	Trace	1.381 ± 0.0007
2	0.463	1.122	Trace	1.585
3	0.441	0.911	None	1.352

TABLE III

NITROGEN ANALYSES OF PLANTS HARVESTED APRIL 27, 1932, 7½ MONTHS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	TOTAL N
	%	%	%
Blades			
1	0.130 ± 0.0009	1.707 ± 0.008	1.837 ± 0.009
2	0.099 ± 0.001	1.573 ± 0.022	1.673 ± 0.020
3	0.148 ± 0.002	1.923 ± 0.008	2.071 ± 0.005
4	0.231 ± 0.007	2.285 ± 0.026	2.516 ± 0.018
5	0.161 ± 0.001	2.139 ± 0.023	2.300 ± 0.024
Stems			
1	0.666 ± 0.019	1.334 ± 0.015	2.000 ± 0.001
2	0.633 ± 0.013	1.294 ± 0.008	1.927 ± 0.022
3	0.344 ± 0.007	1.114 ± 0.002	1.459 ± 0.010
4	0.199 ± 0.002	1.205 ± 0.011	1.404 ± 0.013
5	0.155 ± 0.0007	0.866 ± 0.016	1.021 ± 0.016

of the total blades of series 3 was 36.3 gm., and of series 1, 66.4 gm. At that time the blades of series 1 contained 0.0465 gm. of amino nitrogen per plant, while those of series 3 contained 0.0838 gm. Although weighing only half as much as those of series 1, the blades of series 3 contained almost double the amount of amino nitrogen. This seems to be evidence of a derangement in the synthesis of proteins by the plants deficient in potassium.

TABLE IV

TOTAL AMOUNTS OF PROTEINS WITHIN THE PLANTS

SERIES	AVERAGE DRY WEIGHT OF PLANT		TOTAL PROTEIN N PER PLANT		TOTAL AMINO N PER PLANT	
	NOV.	APRIL	NOV.	APRIL	NOV.	APRIL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Blades						
1	66.4	189.7	1.052	3.238	0.0465	0.2466
3	36.3	69.7	0.530	1.338	0.0838	0.1032
Stems						
1	13.5	299.4	0.133	3.994	0.0533	2.660
3	4.6	36.0	0.030	0.401	0.0203	0.124

The percentages of sugars in the plants are given in tables V and VI. Table V shows that two months after starting the plants in the nutrient solutions there was no great difference in the percentages of sugars in the three groups of plants studied. A slightly higher percentage of reducing sugars and lower percentage of sucrose is suggested in the blades of the potassium-deficient plants at that time. Although these differences may be within the limits of experimental error, it is possible that they suggest a tendency when compared with the results obtained later. In April the

TABLE V

SUGAR PERCENTAGES OF PLANTS HARVESTED NOVEMBER 20, 1931, EXPRESSED ON MOISTURE-FREE BASIS

SERIES	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
	%	%	%
Blades			
1	3.230 ± 0.094	3.717 ± 0.224	6.947 ± 0.318
2	4.235 ± 0.099	2.487	6.722
3	3.548 ± 0.066	3.183 ± 0.074	6.731 ± 0.005
Stems			
1	4.879 ± 0.079	14.630 ± 0.465	19.509 ± 0.385
2	7.003 ± 0.124	14.163 ± 0.138	21.166 ± 0.013
3	4.155 ± 0.109	13.698 ± 0.011	17.853 ± 0.120

blades of the plants starved for potassium were higher in reducing sugars and lower in sucrose than those of the controls, although the percentages of total sugars remained about the same. The stems of series 2 were higher in reducing sugars and lower in sucrose than the controls, but the stems of

the other potassium-deficient plants were very low in reducing sugars as well as sucrose and there was a positive correlation between the amount of potassium supplied and the total sugar produced. These results indicate derangements in the transformations between the hexoses and sucrose. These derangements were found to be correlated with a weak activity of invertase, which will be discussed later.

3. Invertase studies

In a former paper (**24**) evidence was presented indicating that a deficiency in potassium resulted in a weakened activity of invertase in sugar cane. This has been substantiated and studied in greater detail in the present investigation and a preliminary report of some of the results has already appeared (**25**).

TABLE VI

Sugar percentages of plants harvested April 27, 1932, expressed on moisture-free basis

Series	Reducing sugars	Sucrose	Total sugars
	%	%	%
Blades			
1	4.580 ± 0.050	4.225 ± 0.145	8.810 ± 0.095
2	4.360 ± 0.028	5.622 ± 0.014	9.982 ± 0.014
3	5.617 ± 0.134	2.559 ± 0.212	8.176 ± 0.078
4	5.973 ± 0.188	3.070 ± 0.044	9.044 ± 0.145
5	5.346 ± 0.087	3.647 ± 0.005	8.993 ± 0.081
Stems			
1	8.538 ± 0.045	26.220 ± 0.112	34.758 ± 0.157
2	10.055 ± 0.231	21.431 ± 0.053	31.487 ± 0.117
3	8.024 ± 0.057	23.116 ± 0.257	31.140 ± 0.314
4	3.483 ± 0.049	23.747 ± 0.472	27.230 ± 0.522
5	3.653 ± 0.160	18.980 ± 0.160	22.634 ± 0.000

Determinations were made of the activity of the invertase of the blades and the stems of the material harvested in November and in April, both unbuffered and at the optimum reaction. The buffers used in these experiments were those of McIlvaine which are described by Clark (**8**). These were chosen because they do not contain potassium. Controls were run on the hydrogen ion concentration of the tests before and after inversion, which showed that the buffers were efficacious. It was also found that the reaction of the unbuffered tests did not change materially during inversion.

Determinations were made of the effect of the hydrogen ion concentration upon the activity of invertase of the plants of series 1, the results of which are given in table VII.

The results of all the invertase tests are reported as cc. N/20 $KMnO_4$, the Bertrand titration method having been used. With the exception of the effect of the reaction on the invertase activity of the blades harvested in

TABLE VII

pH AND INVERTASE OF SERIES 1

pH	Inversion by H+	Inversion by invertase			
		November		April	
		Blades	Stems	Blades	Stems
		cc.	*cc.*	*cc.*	*cc.*
3.3	7.77			23.30	
3.7	4.71	17.9	52.30	29.91	21.07
4.0	1.83	18.9	59.87	34.09	23.21
4.4	0.90	18.6	62.11	38.27	25.54
4.9	1.39	18.25	49.85	28.87	17.47
5.3	1.15	17.5		20.31	
5.9		16.9			

November, all the tests were conducted in duplicate, with duplicate controls for each lot of material and for each buffer, and were repeated. Table VII shows that the optimum reaction for the invertase of the controls was about pH 4.4.

Invertase determinations of all the plants are presented in table VIII. The determinations of the activity in the blades and stems, buffered and unbuffered, were performed on separate days. While every effort was made to keep the conditions uniform, identical conditions were impossible of attainment. Consequently the results in table VIII are only roughly comparable vertically. They are intended to be read horizontally.

Table VIII shows that the blades and stems of the plants supplied with potassium had a greater invertase activity than those of the potassium-deficient plants. The difference was greater in the stems than in the blades. Greater differences would have been obtained in the stems of the April material if a distinction had been made between green and dry leaf cane, a point which will be discussed later. When tested at the optimum reaction, the activity was apparently equal in all the blades. The activity of the stems was not equalized at the optimum reaction, however, although the difference between the extremes was less than when tested unbuffered.

Because of the importance of hydrogen ion concentration in invertase activity, which is illustrated in table VII, one might suppose that the dif-

TABLE VIII

INVERTASE ACTIVITY IN SUGAR CANE

TEST	SERIES						
	1	2	3	4	5	6	7
	November material						
Blades (unbuffered)	42.00	.....	39.10	28.25	33.89		
Blades pH 4	21.9	21.3	20.16	22.5	22.5		
Stems (unbuffered)	56.45	33.10	16.68	18.65	19.97		
Stems pH 4.4	50.70	42.04	27.42	26.67	22.62		
	April material						
Blades (unbuffered)	25.00	21.21	22.64	17.11	17.71	.....	.....
Blades pH 4.4	36.08	36.16	40.64	39.43	36.69	35.19	34.77
Stems (unbuffered)	22.16	15.64	24.10	10.52	7.97	.....	.....
Stems pH 4.4	23.04	14.82	19.78	15.78	13.57	.....	.....

ferences in activity shown in table VIII were caused by differences in reaction of the unbuffered plant material. This is not true. Colorimetric determinations of the hydrogen ion concentration of the blade and stem powder were made and no correlation was found between the reaction of the powder and the activity of invertase, as will be seen in table IX. For example, in the April material the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction, while series 3 and 4 had the same reaction but differed in activity. In the stems, series 1 had the least favorable reaction but that material was one of the strongest in invertase activity. It is evident that hydrogen ion concentration is not the only factor governing the activity of invertase.

Another factor affecting the activity of invertase in sugar cane is the amount of potassium present in the tissue. The stem powder of the plants of series 1 contained 1.504 per cent. potassium, while that of series 5 had only 0.278 per cent. potassium. An attempt was made in the study reported in 1929 (**24**) to equalize the potassium in the two lots of material; the result was an equal increase in activity in both the control and

TABLE IX

PH OF AIR-DRY POWDER

SERIES	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
	pH	*pH*	*pH*
1	5.6	5.2	5.7
2	5.6	5.2	4.6
3	5.6	5.4	4.6
4	5.8	5.4	4.8
5	5.8	6.0	4.9
6		5.3	5.2
7		5.4	4.9

the potassium-deficient powder. It was concluded at that time that potassium is essential for the formation of invertase within the plant. Unfortunately no buffered tests were made. In the present study the activity of the stems of series 1 and 5 was determined at pH 4.4, with and without the equalization of potassium. The results are reported in table X. The potassium content of the stem powder of these plants was approximately equalized by the addition of potassium dihydrogen phosphate to series 5 and sodium dihydrogen phosphate to series 1. It will be seen that the addition of sodium phosphate to series 1 had no effect, while the potassium phosphate increased the invertase activity of series 5. These data indicate that potassium is a specific accelerator for the activity of invertase in sugar cane, and that sodium and phosphorus are not.

TABLE X

POTASSIUM AND INVERTASE OF STEMS AT PH 4.4

SERIES	POTASSIUM	ACTIVITY ALONE	ACTIVITY WITH EQUAL POTASSIUM
	%		
1	1.504	17.44	18.07 (+Na)
5	0.278	10.85	13.88 (+K)

When all the tests for stems are averaged it is found that when tested unbuffered, series 1 is 2.78 times as active as series 5; at the optimum reaction, series 1 is only 1.71 times as active as series 5; and when the potassium is equalized at the optimum reaction, series 1 is only 1.31 times as active as series 5. Evidently the invertase is present within the potassium-deficient plants, but it needs the proper conditions for its optimum activity.

The plants deficient in potassium showed derangements in the transformations of the sugars correlated with the weak activity of invertase. This

should be expected since invertase is the enzyme which catalyzes the hydrolysis and synthesis of sucrose. Direct evidence for the latter has recently been obtained by OPARIN and KURSSANOW (39). The idea occurred that other factors which curtail the production of sucrose from the hexoses within the plant might operate by their effect upon the activity of invertase.

To obtain additional evidence on this point a supplementary experiment was performed. At the Waipio Substation of the Hawaiian Sugar Planters' Association there was a field of variety P.O.J. 2878 which had received uniform fertilizer and irrigation treatment. For some undetermined reason the juices in the cane in one portion of the field were consistently low in sucrose, while in another part of the same field juices high in sucrose were obtained. The object of the experiment was to determine the juice quality and invertase activity of the plants giving good and poor juices. Five plants of each lot were taken and were subdivided into blades, green leaf cane, and dry leaf cane. The quality of the juices is given in table XI, these data being supplied by the Department of Sugar Technology of this Station.

The invertase activity of these samples is given in table XII. The term green leaf cane is applied to that portion of the millable cane to which green leaves are attached, while dry leaf cane is the part which bears dry leaves or none.

TABLE XI

JUICE QUALITY OF CANE

SAMPLE	BRIX*	POLARIZATION*	PURITY*	QUALITY RATIO*
Good cane (green leaf part)	13.60	9.60	70.6	16.41
Poor cane (green leaf part)	12.89	7.23	56.1	28.73
Good cane (dry leaf part)	22.12	20.30	91.8	6.39
Poor cane (dry leaf part)	20.87	18.84	90.3	6.95

* Purity is the ratio between the polarization (or total sucrose) and the Brix (or total solids measured by the Brix hydrometer), while quality ratio is the number of tons of cane necessary to make one ton of sugar.

It will be seen that when tested unbuffered, the activity of the stems of the cane giving good juices is the same as that of the poor cane. The difference between the activities of the blades of the two lots of plants is not great, but may be significant. The most striking point brought out by this

TABLE XII

INVERTASE ACTIVITY, VARIETY P.O.J. 2878

SAMPLE	CANE GIVING GOOD JUICES	CANE GIVING POOR JUICES
Blades	12.20	9.37
Green leaf cane	12.71	13.99
Dry leaf cane	6.07	6.88

experiment is the fact that the activity in the green leaf part of the stem is double that of the dry leaf portion. This applies to both the good cane and the poor. In the good cane the invertase of the blades is equal in activity to that of the green leaf portion of the stalk. It is evident that wherever sucrose is actively made or stored, invertase activity is great; whereas in the dry leaf portion of the stick, where the storage of sucrose is practically completed, the invertase activity decreases.

4. AMYLASE STUDIES

In the study reported in 1929 (**24**) the activity of amylase was found to be greater in the plants deficient in potassium than in the controls, in both blades and stems. It was suggested that the cause of the increased activity in the plants deficient in potassium might be their greater percentage of sugars, which perhaps constitute the substrate necessary for the formation of starch by amylase, since the amount of substrate has been found in certain cases to affect the amount of enzyme produced. Another possibility suggested was an increase in the absorption of phosphorus in the potassium-deficient plants, since phosphorus is known to favor the activity of amylase, and JOHNSTON and HOAGLAND (**31**) found that tomato plants absorbed an increased amount of phosphorus when the supply of potassium was deficient.

In the present studies, determinations were made of the optimum reaction for the amylase of cane; of the activity in the blades and the stems of the plants supplied with varying amounts of potassium; and of the effects of potassium, phosphorus, calcium, magnesium, dialysis, and sugars upon the activity of amylase. The results are given as color with iodine-potassium iodide after incubation for 20 hours at 36.5° C., using soluble starch as the medium. The following twelve colors are used to represent the course of the digestion of starch, in the order named: blue, purple-blue, purple, red-purple, very dark red, dark red, red, bright red, orange-red, orange, pink-yellow, yellow.

TABLE XIII

pH AND AMYLASE OF SERIES 1

pH	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
2.2	Purple-blue		
4.9	Dark red	Dark red	Red-purple
5.3		Dark red	Red
5.9	Bright red	Bright red	Red
6.3		Red	Red
7.1		Red-purple	Red
8.1	Red-purple		

The effect of the reaction of the medium upon the amylase activity is shown in table XIII. The optimum reaction for the amylase of blades was found to be pH 5.9. The amylase of stems did not seem to be influenced appreciably by hydrogen ion concentration.

The amylase activity of all the plants is given in table XIV.

TABLE XIV

AMYLASE OF PLANTS GROWN WITH VARYING AMOUNTS OF POTASSIUM

SERIES	NOVEMBER MATERIAL		APRIL MATERIAL	
	BLADES (pH 5.9)	STEMS (UNBUFFERED)	BLADES (pH 5.9)	STEMS (UNBUFFERED)
1	Very dark red	Orange-red	Red-purple	Red-purple
2	Dark red		Red	Purple
3	Orange	Yellow	Orange	Red
4	Yellow	Yellow	Yellow	Orange
5	Pink-yellow	Yellow	Orange-red	Bright red
6			Dark re..	Dark red
7			Dark red	Dark red

The amylase activity of the blades of the November material in decreasing order was $4 > 5 > 3 > 2 > 1$. That of the blades of the April material was $4 > 3 > 5 > 2 > 6$ and $7 > 1$. That of the stems collected in April was $4 > 5 > 3 > 6$ and $7 > 1 > 2$. In every test the plants deficient in potassium had greater amylase activity than the controls.

Studies were made concerning the cause of the active amylase of the potassium-deficient plants:

a. Sugars

Perhaps the formation of starch from dextrose in plants is catalyzed by amylase. If so, and if the production of amylase is regulated by the amount of dextrose, then the plants which have the most active amylase should also contain the largest percentage of reducing sugars, other things being equal. The data for the sugars in the November harvest are insufficient for this comparison. Table VI shows that the percentage of reducing sugars in the blades of the April material decreased in the order $4 > 3 > 5 > 2 > 1$. The amylase of the same blades differed in the same order. The stems of the plants deficient in potassium, however, had the lowest percentage of reducing sugars and the most active amylase. In short, although the principle of the quantitative regulation of enzyme production might apply to the amylase activity in the blades, it could not be applied in the stems.

Before the sugar determinations were performed a supplementary test was conducted to determine the effect of sugars upon the activity of amylase. Cane shoots were taken from cuttings and supported in split corks in Erlenmeyer flasks, and were placed in intense diffused light. One plant was given distilled water, another a 1 per cent. solution of sucrose, and a third a 1 per cent. solution of dextrose. The sugars and water had been boiled and cooled. They were renewed at intervals. After four days the blades were removed, ground and dried, and amylase determinations were performed. At the time of the removal of the blades the three plants appeared about the same, except that the spindle of the plant which had received dextrose was greener than the spindles of the other two plants. It was found that the plant which had received dextrose had the most active amylase, that which had been in water was the least active, while the amylase of the plant receiving sucrose was intermediate in activity. These results are not in agreement with those of SJÖBERG (**43**), who found that bean plants receiving sugars had weaker amylase activity in stems and leaves than those with no organic nutrients. The present results seem to uphold the general theory of the effect of the substrate upon the production of enzymes, although, as explained above, that theory cannot explain the amylase activity in the stems in these potash investigations.

b. Phosphorus

The percentages of phosphorus in the plants were given in tables XV and XVI of the first paper of this series (**26**). The relative amounts of phosphorus, potassium, and amylase are given in table XV, in which the

TABLE XV

RELATIVE AMOUNTS OF PHOSPHORUS AND POTASSIUM, AND AMYLASE ACTIVITY

	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
Phosphorus	5 > 4 > 3 > 2 > 1	4 > 5 > 3 > 2 > 1	5 > 4 > 1 and 3 > 2
Potassium	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 4 > 5
Amylase	4 > 5 > 3 > 2 > 1	4 > 3 > 5 > 2 > 1	4 > 5 > 3 > 1 > 2

figures are the series numbers of the plants. From this it will be seen that there is a fairly good positive relationship between the phosphorus content and the amylase activity. There is also a good negative correlation between the potassium content and the amylase activity.

Tests were conducted with the blades of the material collected in both November and April to determine whether equalizing the phosphorus would result in the equality of the activity of amylase and it was found that it did not. Evidence was obtained, however, indicating that the amylase of the potassium-deficient blades is activated by phosphorus more than is the amylase of the control blades, as shown in table XVI.

TABLE XVI

AMYLASE ACTIVATION BY PHOSPHORUS

SERIES	TREATMENT	COLOR WITH IKI
Blades		
1	H_2O	Dark red
1	Na phosphate	Dark red
2	H_2O	Orange-red
2	Na phosphate	Orange

c. Theories more or less disproved

Aspargine is known to be an activator of amylase. A microchemical test was made of the blades of series 1 and 4, using copper acetate. No marine blue crystals of copper asparginate were found, indicating that little or no asparagine was present.

Sodium is one of the ions known to activate amylase, but the plants of series 5 were grown entirely without sodium and their amylase was about equal in activity to the amylase of series 4.

It might be supposed that the potassium content of the control plants was high enough to exert a direct inhibitory action upon amylase. This

was not true because the addition of potassium to the blades of the control plants harvested in November resulted in a slight increase in activity.

OPARIN and DJATSCHKOW (38) concluded from studies with wheat that amylase is made in the plant and goes to the grain. Amylase might be made in the blades of the sugar cane plant, its production being controlled by the sugar content of the blades rather than of the stems. But if amylase migrates from the blades to the stems it would supposedly go through the phloem. The phloem necrosis which curtailed the translocation of proteins and sugars from the blades to the stems would also prevent the migration, of amylase. This should tend to result in less amylase in the stems of the potassium-deficient plants than in the controls, which was not found.

d. Activation by salt constituents in general

The blades of the potassium-deficient plants harvested in November were higher in total ash, calcium, and magnesium than were the controls, as shown in the first paper of this series (26). These differences held for magnesium in the blades and stems of the April material, and for calcium in the blades, but did not hold for calcium in the other organs or for total ash. Therefore, although the higher ash, calcium, and magnesium content of the potassium-deficient plants might explain their more active amylase in the material of the November harvest, only the magnesium content could apply to that collected in April.

The magnesium contents of the blades of series 1 and 2, harvested in November, were equalized by the addition of magnesium sulphate to the former. It was found that the magnesium sulphate activated the amylase of the blades of series 1 slightly but did not make it equal to that of series 2. Similar results were found when the calcium content was equalized.

To find the effect of the ash constituents in general, the blades of the November harvest were dialyzed in collodion bags before incubation. Pre-

TABLE XVII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 24 HOURS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Dark red	Red-purple
2	Red	Dark red
3	Pink-yellow	Orange
4	Yellow	Pink-yellow
5	Yellow	Pink-yellow

liminary tests and controls showed that the amylase of sugar cane is non-dialyzable through collodion. The amylase activity of the blades with and without previous dialysis for 24 hours is shown in table XVII.

In every plant, dialysis for 24 hours resulted in decreased activity, indicating the loss of an activator. In another experiment dialysis was continued for three days. Amylase determinations were then performed, the results of which are given in table XVIII.

Dialysis for three days thus decreased the amylase activity of series 2, 4, and 5 but increased that of series 1 and 3. This shows that dialysis

TABLE XVIII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 3 DAYS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Purple	Red-purple
2	Red-purple	Purple
3	Very dark red	Dark red
4	Pink-yellow	Red
5	Pink-yellow	Dark red

even for three days does not equalize the activity of amylase. It is therefore not the mere presence of the ash constituents which alone determines the activity of amylase. It should not be surprising that dialysis at times results in increased activity of amylase and at other times decreases its action. Among the dialyzable constituents of the plant material it seems only reasonable that there are some which increase and some which decrease the activity of amylase, and that these diffuse at different rates.

Further considerations of the amylase activity will be found in the discussion.

Other tests to be reported in another contribution have shown greater dextrinase and weaker maltase activity in the blades of cane plants deficient in potassium than in the controls. Greater differences in maltase activity were found in wheat and buckwheat than in sugar cane.

5. EREPTASE STUDIES

The optimum reaction for the activity of ereptase was determined and the results are shown in table XIX. The test consisted of the formation of tryptophane from Witte peptone, the amount of tryptophane being de-

termined by the addition of bromine water. This gives a pink color and the deeper the pink the greater the amount of tryptophane, and hence the greater the activity of ereptase.

TABLE XIX

pH AND EREPTASE OF SERIES 1

pH	NOVEMBER MATERIAL		APRIL MATERIAL		
	BLADES	ROOTS	BLADES	STEMS	ROOTS
2.2	Colorless*				
3.3		Colorless	Lightest pink		
3.7		Colorless	Lighter pink		Colorless
4.0		Light pink	Light pink	Colorless	Very light pink
4.4			Pink	Colorless	Light pink
4.9	Light pink	Very light pink	Rose	Colorless	Pink
5.3			Light pink	Light pink	Light pink
5.5				Pink	
5.9	Colorless		-	Deeper pink	Colorless
7.0				Light pink	
8.1	Colorless			Colorless	

* Negative results; blanks indicate no test performed.

The optimum reaction for the blades, November and April, was found to be pH 4.9, stems 5.9, and roots about pH 4.9.

Determinations were made of the activity of ereptase of the blades and roots of the plants harvested in November, and of the blades, stems, and roots of the April material, all the tests being made at the optimum reaction. No difference was found in the activity in the blades. Some slight differences occurred in the stems, but they were not correlated with the amount of potassium supplied nor with any other observed factor. The activity in the roots of the material harvested in April occurred in the order $4 > 3$ and $5 > 2$ and $7 > 1 > 6$. The gradation is not perfect, but it does indicate greater activity in the plants deficient in potassium.

6. PEPTASE STUDIES

Peptase activity was found in the studies reported in 1929 (**24**) to be greater in the controls than in the plants deficient in potassium. Since

peptase may be important in catalyzing the synthesis of proteins, any derangement in its activity might be expected to reduce the production of protoplasm and hence curtail growth.

It was hoped to repeat the studies of peptase in the present investigations, using a more exact method. To date no evidence of peptase activity has been obtained. Using the carmine fibrin method of GRÜTZNER (**22**), no peptase activity was detected in blades or roots at pH 2.2–5.9. With the titration of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) no proteolytic action was demonstrated at pH 2.2, 3.3, 4, 4.9, 7.1, or 8.1. In these tests water was used as the medium for extraction. According to VINES (**51**) ereptase is generally more readily extracted by water while peptase is extracted more easily by 2 per cent. NaCl. However, where plants have been grown in a concentrated nutrient solution, VINES found that peptase could be extracted with water. Inasmuch as the nutrient solutions used in the Chicago experiments were much more concentrated than those used in the present studies, the idea occurred that the peptase of the former plants might have been more readily soluble in water than that of the present investigation, which might require additional salt for extraction. To determine this point, blade material was extracted for two days in 2 per cent. NaCl. The reaction was then adjusted to pH 5.2–5.3 with sodium dihydrogen phosphate, and a peptase test was performed using carmine fibrin. No sign of digestion occurred. The WILLSTÄTTER-WALDSCHMIDT-LEITZ method was employed at pH 4.9 and 5.4, with negative results.

The conclusion is drawn that under the conditions of the present investigations, no peptase was found. Possibly using a different method of extraction or a different hydrogen ion concentration, positive results would be obtained. Perhaps the more concentrated nutrient solutions used in the former study (**24**) caused the development of peptase strong enough to be detected. Peptase is probably present in cane but in such small amounts that it is often undetectable. For the study of the relation of potassium to the activity of peptase, soy beans or other plants high in proteins might be more desirable.

Notwithstanding the fact that no evidence of peptase was obtained, derangements in the formation of proteins were indicated. Growth was proportional to the amount of potassium supplied. In November the dry weight of the blades of series 3 was about half that of series 1, whereas series 3 contained nearly double the amount of amino nitrogen, as shown in table IV. This would seem to indicate difficulty in some step of the synthesis of proteins. Because the activity of ereptase was equal in all the blades, the indications are that the synthesis of the higher proteins was curtailed by the deficiency of potassium. Whether or not this was due to a weak activity of peptase as shown previously (**24**) could not be ascer-

tained in the present study. The analogy between the nitrogen and sugar data is interesting although inconclusive.

Discussion

Since the chemical transformations in plants are chiefly catalyzed by enzymes, it would seem that quantitative determinations of their activity in connection with chemical analyses in studies of mineral deficiencies might lead to important results. A few studies of the effects of potassium upon the activity of enzymes will be mentioned. DOBY and HIBBARD (**10, 11**) found more active invertase and diastase in sugar beets deficient in potassium than in those supplied with that element. HARTT (**24**) reported derangements in the activities of invertase, diastase, peptase, and catalase in sugar cane plants grown without potassium. ECKERSON (**12**) found that plants low in potassium are weak in reducase activity. In the present studies a decreased activity of invertase and increased activity of amylase were found in the cane plants deficient in potassium. It would seem that these derangements in enzyme action might be closely connected with the disturbance in the protein and carbohydrate metabolism of the plant.

While it is probably not essential to consider that all of the reactions catalyzed by enzymes are reversible, yet this has been proved for several and assumed for many. The supposition that there is a separate enzyme which condenses dextrose and levulose to form sucrose does not seem necessary in view of the recent work of OPARIN and KURSSANOW (**39**), who have demonstrated the synthetic action of invertase. Their report provides a summary of the subject of enzymatic synthesis. In the studies, the views that sucrose is not the first sugar formed in photosynthesis, and that both the synthetic and the analytic reactions are catalyzed by the same enzymes, are taken as working hypotheses. It is realized that further studies may disprove these points, but at present they seem to explain the results obtained.

The enzymes of sugar cane have been insufficiently studied. BROWNE (**4**) mentioned the presence of the following enzymes in cane: diastase, invertase, oxidase, and a reducing enzyme. In addition to these, BROWNE and BLOUIN (**5**) stated that peptonizing enzymes are present in cane stalks. HARTT (**24**) reported the results of quantitative determinations of diastase invertase, peptase, ereptase, and catalase. NEEB (**36**) reported the occurrence of saccharase, amylase, catalase, tryosinase, oxidase, peroxidase, and maltase.

PROTEIN METABOLISM

The subject of the utilization of potassium by plants, with particular reference to the interrelationships between carbohydrate and protein

metabolism in plants deficient in potassium, has been so well summarized by NIGHTINGALE *et al.* (**37**) in connection with studies of the tomato plant that it does not need to be repeated here. In substance, they conclude that where carbohydrates accumulate in potassium-deficient plants, a principal cause of their accumulation is a derangement in the synthesis of proteins. A similar conclusion had previously been reached by HARTT (**24**), who found higher percentages of total sugars and increased formation of lignin correlated with a deficiency in the activity of peptase in sugar cane plants grown without potassium.

In the present studies, derangements in the synthesis of proteins in the plants deficient in potassium were indicated indirectly by the curtailment of growth (**26**) and directly by the nitrogen determinations. Two months after starting the plants in the nutrient solutions, the blades of series 3 had a higher percentage of amino nitrogen and a lower percentage of protein nitrogen than those of series 1, the total nitrogen remaining about the same. Similar though less pronounced differences were found in the stems, as shown in table II. Not only was there a difference in percentages, but the actual amount of amino nitrogen in the blades of series 3 was almost double that in series 1, although the average dry weight of the plants of series 1 was about twice that of those of series 3, as shown in table IV. This accumulation of amino nitrogen in the blades and stems of the plants deficient in potash which occurred at the age of two months, together with the slight decrease in percentage of protein nitrogen, seems to be evidence of the failure of the potassium-deficient plants to synthesize proteins as usual. Attention is called to the fact that the curtailment in synthesis occurred after the formation of amino acids rather than before, indicating that in these plants the reduction of nitrates proceeded as usual. Notwithstanding this point, reducase determinations would be interesting and would have been made had time permitted.

The terminology of proteolytic enzymes has become considerably complicated in recent years. Following VINES (**51**), in this paper the simple distinction between peptase and ereptase is made, the former catalyzing the digestion of complex proteins to peptones and allied compounds, and the latter carrying the digestion from these intermediate products to amino acids. Since there is little evidence of a tryptic enzyme occurring generally in plants, that is left out of consideration. In the studies reported in 1929 (**24**) both peptase and ereptase were found to occur in cane, whereas in the present study ereptase was the only proteolytic enzyme detected. As mentioned in the results, the use of other solvents at other reactions might have shown the presence of peptase. Inasmuch as the activity of ereptase seemed not to be affected by the supply of potassium, with the possible exception of the roots, derangements in ereptase cannot explain the deficiency

in the synthesis of proteins. If the synthesis of proteins follows the same steps as their digestion, would not a weakened peptase activity result in the accumulation of peptase and proteoses rather than amino acids? It is, however, conceivable that both might occur. It is unfortunate that no determinations of the forms of nitrogen were made in the Chicago studies, and that the attempted detection of peptase in the Honolulu studies failed. Since at the age of two months the synthesis of proteins in the potassium-deficient plants had proceeded as usual, as far as the production of amino nitrogen, it seems likely that either some essential amino acids were lacking or that a derangement occurred in the activity of the higher proteolytic enzymes. There is need for further work along these lines.

Although in November the stems of the plants deprived of potash were slightly higher in amino nitrogen and lower in protein nitrogen than were the controls, in April the stems of the potassium-deficient plants had considerably lower percentages of amino, protein, and total nitrogen than the controls. The blades of the plants harvested in April showed higher percentages of amino, protein, and total nitrogen in the plants deficient in potassium than in those supplied with an adequate amount of that element. Thus the proteins of the blades and stems of the plants harvested in November varied in the same way, while in the April harvest the results of the protein analyses of the blades and stems were diametrically opposed. These observations may be explained by assuming that the chief seat of synthesis of amino acids and simple proteins is in the blades; that at least some water and nutrient salts can still move up in the xylem of the potassium-deficient plants; but that a necrosis of the phloem curtailed the translocation of nitrogenous compounds from the blades to the stems. Evidence of phloem necrosis possibly similar to that reported by JOHNSTON and DORE (**30**) has been presented in the first paper of this series (**26**), the evidence consisting of brown discolorations of the sieve tubes and companion cells in the midribs and stems of the plants deficient in potassium. The necrosis might be due either to a disturbance in the synthesis of proteins resulting in abnormal protoplasm in the sieve tubes and companion cells, or to the accumulations of iron at the nodes resulting in the coagulation of the proteins of the phloem, or to both conditions. Whatever the cause, it is apparent that such a necrosis might seriously interfere with the translocation of nitrogenous and carbohydrate compounds.

In short, the results here reported indicate that both the synthesis and the translocation of proteins in the sugar cane plant are decreased by a deficiency in potassium. The derangement in the synthesis of proteins is possibly caused by a weak activity of peptase, as shown by former studies (**24**). The interference with their translocation may be caused by the necrosis of the phloem.

Differences so slight as to be within the limits of experimental error in the November harvest became intensified by the time of the April harvest, when the blades of the plants deficient in potassium had greater percentages of reducing sugars and lower percentages of sucrose than the controls, as shown in table VI. Inasmuch as the activity of invertase was greater in the plants receiving potassium than in those deprived of that element, it would seem that invertase aids in the synthesis of sucrose in the blades of sugar cane.

In the stems of the plants harvested in April a distinction must be made between partial and complete starvation for potassium. The plants of series 2, which were only partially starved for potash, reacted the same way as the blades, having a higher percentage of reducing sugars and lower percentage of sucrose than the plants of series 1. The other plants were more severely affected, the percentages of reducing sugars, sucrose, and total sugars all being lower in series 3, 4, and 5 than in series 1. The activity of invertase was weaker in the plants starved for potash. The supply of dextrose, the essential source of energy for growth and life, was curtailed especially in the stems of series 4 and 5, both by the necrosis of the phloem which would interfere with the translocation of sugars from the blades to the stems, and by the weak activity of invertase which would decrease the inversion of the sucrose already in the stems.

The suggestion is therefore made that a deficiency in potassium in the sugar cane plant may interfere with the transformations between the hexoses and sucrose, both anabolic and catabolic, as well as with their translocation.

From the practical standpoint the object of the sugar planter is to obtain the plant which stores the most sucrose in the stems. Under field conditions it is unlikely that any plants as deficient in potassium as the plants of series 3, 4, and 5 would continue until harvest. Their weakened condition is so obvious that further applications of fertilizer would be made, or else they would probably die. The important distinction for the planter to make is between plants such as series 1 and 2, those plants which appear very much the same in color, size, and general condition but which show a difference in the amount of sucrose in the stems. The stems of series 2 were higher in reducing sugars and lower in sucrose than those of series 1, and the activity of invertase in series 2 was weaker than that of series 1. If plants in the field similar to the plants of series 2 could be recognized in time and given the proper fertilizer, it seems possible that the activity of their invertase could be increased and so increase the amount of sucrose stored in the stems.

The relationship between potash fertilization and yield is obscure. VAN DEN HONERT (**50**) found a low figure for Brix in variety P.O.J. 2878 deficient in potassium, but suggested that this might be caused by the fact that the suckers of the plants starved for potash were younger and less mature than the controls. As mentioned in the discussion of the first paper in this series (**26**), the results of some experiments have shown an increase in yield of sugar following the application of potash, others have shown no response, and still others have indicated a depressing effect, the last condition possibly resulting from a depression in the absorption of phosphorus. It is evident that a delicate relationship exists. While it is by no means suggested that the only factor involved is the activity of invertase, yet the present results show that enzyme to be important, and it is felt that further attention should be paid to the activity of invertase in determining the effects of fertilizer applications and other cultural practices. This matter is discussed more fully below.

For a complete understanding of the carbohydrate metabolism of potassium-deficient plants, certain fundamental principles should be determined. Is sucrose synthesized in the leaves by the enzyme invertase? Is sucrose merely a temporary storage product in the leaves, converted into hexoses before translocation to the stems, or is sucrose the main translocation form of sugar, or are both the hexoses and sucrose translocated? These questions being of general importance are summarized in textbooks of plant physiology. The investigations with sugar cane have been discussed by VISWANATH (**52**), who concluded that the bulk of evidence seems to favor the view that sucrose is built up in the stem from reducing sugars sent into it by the leaf. GEERLIGS (**19**), however, is of the opinion that sucrose, dextrose, and levulose are all continuously supplied by the leaf.

The writer is now conducting studies on these points and the results will be reported later. In the meantime it would seem that the present studies offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase, as already mentioned in a preliminary report (**25**). The blades of the plants deficient in potassium had the highest percentage of reducing sugars and lowest percentage of sucrose, and they also had the weakest activity of invertase. Since the percentage of total sugars remained about the same in the blades, it would seem that this is evidence of a derangement in the forms of sugars rather than a difference caused by some other factor, such as photosynthesis or protein formation. Interference with translocation may have affected the results in the material harvested in April, since both sucrose and hexoses were low in the stems of the plants deficient in potash, with the exception of the plants of series 2. If the action of invertase is only hydrolytic, then what is the explanation of the greater percentage of reducing sugars and lower percentage of sucrose in the plants

which had the weakest invertase activity? Further evidence of the importance of invertase in the synthesis of sucrose is found in the supplementary experiment using variety P.O.J. 2878, described earlier in this paper. Millable cane may be divided into two physiologically different regions: the green leaf cane (or that portion which bears green leaves) and the dry leaf cane (the part bearing dry leaves or none). It is in the green leaf portion of the stem that the most active storage of sucrose occurs, although some additional storage takes place in the dry leaf cane. As shown in table XII, the invertase activity of the green leaf cane was found to be double that of the dry leaf cane. The invertase of the blades was found to be equal in activity to that of the green leaf cane. It is evident that wherever sucrose is actively formed or stored, the activity of invertase is strong, whereas where the process of storage is practically completed, invertase decreases in activity.

The question of the accumulation of carbohydrates in plants deficient in potassium may next be considered. This has already been discussed with references to the literature in an earlier paper (**24**) and also by NIGHTINGALE (**37**). In the present study no evidence of carbohydrate accumulation was obtained. In the blades the total sugars were about the same in all five groups, while in the stems of the plants harvested in April there was a positive correlation between the amount of potassium supplied and the percentage of total sugars formed. As pointed out by NIGHTINGALE, the accumulation of carbohydrates may be an early condition in plants deficient in potassium, disappearing later. Inasmuch as the plants of the present study grew so much more rapidly than those of the former investigation, it may be that at the time of the first harvest the period of carbohydrate accumulation had already passed, if any occurred at all.

It has been shown so far that the utilization of potassium by the sugar cane plant involves the synthesis and translocation of both proteins and carbohydrates. Potassium may affect the synthesis of proteins through its effect on the enzyme peptase, as shown previously (**24**). Potassium affects the transformations of the sugars through its effect on invertase. The question of how potassium affects invertase may next be considered.

Various possibilities suggest themselves as to how potassium affects the activity of invertase: (1) the relationship may be direct, through its entering into the chemical composition of the enzyme, or through its effect as a specific activator; (2) it may be indirect, regulating the hydrogen ion concentration, or the buffer system; (3) it may be antagonistic, in offsetting the effects of inhibitory factors. In a previous report (**24**) the importance of potassium in the formation of invertase was stressed; those tests, however, were all unbuffered. Inasmuch as the activity of invertase in all the blades was equalized at the optimum reaction, it is now felt that potassium is not

essential for the formation of invertase. Because of the equalization of activity at the optimum reaction, it might be concluded that the differences in invertase activity in the blades are due solely to differences in hydrogen ion concentration. This is erroneous since, as shown in table IX, the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction; while those of series 3 and 4 had the same reaction but differed in activity. Regarding the possibility of there being differences in the buffer systems, this matter was considered, and it will be seen from figure 1 that little if any difference was found to exist. The suggestion that potassium may offset the effects of inhibitory factors, particularly salt constituents, deserves consideration since, as shown by FALES and NELSON (**14**), the addition of sodium chloride had practically no effect on the velocity of the hydrolysis of sucrose by yeast sucrase at the optimum reaction; but at all other reactions the salt inhibited the action of the enzyme. The greater ash content of the potassium-deficient plants harvested in November might inhibit invertase, but this explanation could not apply to the April material, because in that harvest the plants of series 1 and 2 had the greatest ash content.

Thus either there was some undetermined inhibitory factor, or potassium is a specific activator for invertase in sugar cane, in which latter case the addition of potassium to the enzyme tests should result in increasing the invertase activity; and equalizing the potassium in material from potassium-deficient and control plants should result in approximately equalizing the activity of invertase. Such an experiment was conducted, the results of which are given in table X. Enough potassium dihydrogen phosphate was added to the stem material of series 5 to equalize the potassium in series 1 and 5, and sodium dihydrogen phosphate was added to series 1 to maintain such conditions as hydrogen ion and osmotic concentrations. The test was conducted at pH 4.4. It was found that the addition of potassium to series 5 did not make the activity equal to that of series 1, but did increase it somewhat. As already mentioned, when tested unbuffered, the stem material of series 1 was 2.78 times as active as that of series 5; but when potassium was equalized at the optimum reaction, series 1 was only 1.31 times as active as series 5 (averages of all tests). Since the addition of sodium phosphate to series 1 did not increase the activity, while the potassium phosphate did activate the invertase of series 5, it would seem that potassium is a specific accelerator for the enzyme invertase. If this is true for other plants which store sucrose then it may help to explain the statement of COLIN and BILLON (**9**) that potassium plays a direct rôle in the formation of sucrose in beets.

The preceding discussion shows that the invertase of stems is affected more severely by potassium deficiency than is that of blades. The differ-

ence between the extremes is much greater in the stems than in the blades (table VIII), and the activity in the blades is equalized at the optimum reaction while that in the stems is not. Although a possible explanation is the higher percentage of potassium in the blades than in the stems, as shown in table XVI of the first paper in this series (**26**), yet the subject is too complex to permit complete understanding at the present time.

The accelerating effect of potassium may help to explain the differences in invertase activity in the green leaf cane as compared with that in the dry leaf cane. Table XII shows that the activity in the green leaf cane is double that of the dry leaf cane. Analyses performed by the Chemistry Department of this Station have shown that the percentage of potassium increases from the bottom to the top of the stalk. While other factors undoubtedly contribute to the differences in the activity of invertase in various parts of the stalk, it is suggested that the differences in percentage of potassium are important. Further studies along these lines are now under way.

Certain data in table VIII require discussion at this point. There it will be seen that the invertase of the stems of series 2, April material, is less active than that of series 1 or 3, whether buffered or not. This puzzled the writer until the distinction between green and dry leaf cane became apparent. The plants of series 1 and 2 were very much larger than the others and consequently had considerably more dry leaf cane. Inasmuch as the entire sticks were compounded, the weak invertase of the dry leaf cane would naturally decrease the figures for the entire stalk. This may also explain why the activity of the stems of the controls is so much less in the April than in the November harvest, there being considerably more dry leaf cane in April than in November. The distribution of invertase throughout the cane plant is now being studied and will be reported in a later contribution.

The sensitivity of the invertase of yeast to hydrogen ion concentration is well known and has been discussed by FALK (**15**). The data presented in table VII show that the invertase of sugar cane is similarly affected, having in these plants an optimum reaction very close to that of the invertase of yeast. The effect of hydrogen ion concentration upon the activity of invertase differs from its direct effect upon inversion: in the former case there is a definite optimum around pH 4.4, the activity falling off at other reactions; in the latter case inversion increases with increasing acidity as far as has been tested. The significance of this situation has been examined by FALK, who suggests that the hydrogen ion and the enzyme may attack sucrose differently.

The hydrogen ion concentration of the expressed sap of the blades and stems of the plants harvested December 4 was pH 5.2 to 5.3 (table I), while

the optimum reaction for the invertase of the plants harvested November 20 was pH 4 to 4.4 (table VII). The question arises as to the effect of the hydrogen ion concentration of the expressed sap of cane upon the quality of the juice. In Trinidad, FOLLETT-SMITH (18) found the sap of cane leaves to be pH 5.1–5.3. So far as the writer is aware, these are the first determinations to be made in Hawaii of the hydrogen ion concentration of the expressed sap of sugar cane. It is therefore impossible at present to compare these results with others in Hawaii. Some data are available for the reaction of crusher juices at the Honolulu Plantation Company, where the average of 50 determinations is pH 5.17 for crusher juice and pH 5.29 for mixed juice. Although these figures are close to those obtained in the present study, probably little value should be assigned to them in this connection for two reasons: (1) the crusher juice is contaminated with soil and fertilizers; and (2) the crusher juice is composed largely of the juice from dry leaf cane, which may or may not have the same reaction as green leaf cane. There is need, therefore, for further studies concerning the hydrogen ion concentration of the expressed sap, invertase activity, and juice quality. Several questions arise. Is the optimum reaction for the formation of sucrose the same as the optimum for the analytic activity of invertase? If the synthesis of sucrose is as sensitive to reaction as the activity of invertase is shown to be in this paper, which seems likely from the work of OPARIN and KURSSANOW (39), then studies should be made of the possibility of controlling the hydrogen ion concentration of the cell sap of sugar cane.

The invertase studies reported here show that the following factors affect its activity: potassium, hydrogen ion concentration, and location in the plant. Inasmuch as enzymes are very sensitive to conditions, it seems likely that many other climatic and edaphic factors may affect its activity. The evidence presented in this paper would seem to indicate that invertase in the sugar cane plant synthesizes sucrose, and that where invertase is most active the greater synthesis of sucrose occurs. For the past 20 years the increase in sugar percentage within the cane plant has not been commensurate with the increase in the applications of fertilizers, although the increase in the total sugar produced per acre has been large because of the greater size of the plants, a point which has been discussed by MARTIN and MCCLEERY (35). An important problem in the sugar industry today, therefore, is the improvement of the quality of the juices. Juice quality is known to be affected by weather, fertilizers, etc., but the mechanism is not fully understood. It would seem that both external and internal factors may affect the activity of invertase and that a study of these may aid in controlling the production of sugar. Such studies are now under way.

Another important effect of potassium deficiency in sugar cane is the increase in the activity of amylase. A relationship between potassium content and amylase activity has been suggested by several investigators. ROBBINS (42) studied the secretion of diastase by *Penicillium camembertii* and concluded that there was no relationship between potassium and diastase formation. ENGLIS and LUNT (13) reported on the diastatic activity of the nasturtium; they found that the activity of diastase decreased with increased potassium in sand, but in peat the medium application of potassium gave the highest activity. DOBY and HIBBARD (10, 11) found increased diastase activity in sugar beet plants deprived of potash. JAMES (28), from his studies of the effect of potassium upon photosynthetic efficiency in the potato, postulates that potassium increases the activity of diastase. Although for other plants the results are not always in agreement, a more active amylase in potassium-deficient plants seems now to be well established for sugar cane, since the present results are in accord with the former (24).

For the sake of clarity, most of the discussion of the amylase determinations was incorporated with the presentation of results and the reader is referred to that section at this point. The following possibilities arose as explanations of the increased amylase activity in the potassium-deficient plants: the quantitative regulation of amylase production by sugars; the stimulating effect of phosphorus; a direct regulatory or protective effect exerted by potassium. Certain theories almost certainly disproved were mentioned in the results and will not be repeated here.

Although some evidence was obtained in favor of the theory of the quantitative regulation of enzyme production by the substrate, that theory is not of general application in the present study because of the negative relationship between the amylase activity and percentage of reducing sugars in the stems of the plants harvested in April, whereas the correlation should be positive to uphold the theory.

The greater amounts of phosphorus in the potassium-deficient plants may play a rôle in their amylase activity, since in both the November and April material the largest amounts of phosphorus occurred in the plants deficient in potash, as shown in tables XV and XVI of the first report in this series (26). The correlation between phosphorus content and amylase activity is not perfect, however, and the equalization of the phosphorus in the blades of the November and April material did not result in equal activity of amylase. It is interesting, on the other hand, that the amylase of the potassium-deficient plants was activated by the addition of more phosphorus, while that of the control was not. The plants deficient in potassium contained more phosphorus and their amylase was more readily activated by phosphorus. This is indeed an important factor.

On the whole the evidence at hand seems to favor most strongly the idea that potassium acts as a regulator or protector of the enzyme amylase. It must be considered that the activity in the control plants is "normal" while that in the plants deficient in potassium is "abnormal." The suggestion of GOODWIN and HANGER (**20**) is pertinent, that amylase is a negatively charged ion. If so, it might readily unite with the positively charged potassium ion and this might explain the dialysis results. Dialyzing one day might remove the inorganic activators and thus decrease activity, but further dialysis (3 days) might cause or allow the amylase-potassium compound to dissociate, the potassium going through the membrane, leaving the amylase unprotected and thus more active. Possibly the negatively charged amylase is more readily hydrated than the amylase-potassium compound, since the charging of proteins is known to run parallel with hydration. This might aid in the digestion of starch, the first step of which is hydration. Further evidence of the protective effect of potassium is found in the fact that the amylase activity of the potassium-deficient plants was more readily activated by phosphorus than was that of the controls. DOBY and HIBBARD (**10, 11**) also found that the diastase of sugar beets deficient in potassium was activated by salts more readily than that of beets supplied with an adequate amount of potash. There is a negative relationship between potassium and amylase in both the blades and the stems of the material harvested in November as well as in April (table XV). Changing representative plants from solutions lacking potassium to the control solution in January resulted in a decrease in their amylase activity (table XIV). In short, the evidence points to the view that one function of potassium in the sugar cane plant is the regulation or "protection" of the enzyme amylase. Leaf diastase is supposed to consist of an inactive pro-enzyme and an activating co-enzyme, according to WAKSMAN and DAVISON (**53**). Possibly potassium exerts a protective action on the pro-enzyme, thus preventing its activation by the co-enzyme or other accelerators.

The condensations of dextrose in the formation of dextrins and starch tie up the sugar in undesirable forms which may result in decreasing the yield of sucrose. Starch occurs in cane as a temporary storage product in the starch sheaths of the leaves. This starch is probably for the most part digested to sugar and translocated to the stems and stored as sucrose, in the ordinary course of events. In certain varieties, however, a deposition of starch occurs in the stems, particularly in the nodes. This is especially true of Uba canes, a condition which has been studied by FEUILHERADE (**16, 17**), HADDON (**23**), VON STIEGLITZ (**44**), and WELLER (**54**). One is led to wonder whether the amylase activity of Uba is different from that of other varieties.

The production of too much starch and the like in cane is undesirable not only because of the utilization of dextrose which should go to the formation of sucrose, but also because the presence of certain gums, dextrins, and other higher carbohydrates in the juices leads to difficulties in the process of clarification, as brought out by FEUILHERADE and STIEGLITZ.

Amylase and invertase differ in several respects and a consideration of these differences is interesting. Amylase was more active in the potassium-deficient plants whereas invertase was less active. Amylase was found to be much less sensitive to hydrogen ion concentration than was invertase. Invertase was activated by potassium but not by sodium, whereas amylase was activated by everything tested. The two enzymes behaved similarly in that both were more readily activated in the potassium-deficient plants than in the controls.

RÔLE OF POTASSIUM

The question of the rôle of potassium in the nutrition of plants has interested plant physiologists for years, and men have sought to assign one particular function to the element, *e.g.*, photosynthesis, protein synthesis, translocation, or others. The research of recent years, (**3, 21, 24** and **26, 28** and **29, 37,** and others) has shown that the problem is not so simple, but that potassium probably affects directly or indirectly many if not all of the cellular activities of plants. Thus the present studies show that sugar cane plants deficient in potassium may develop the following characteristics: low percentage of moisture; high total ash content when young, followed later by low ash percentage; high percentages of calcium, magnesium, phosphorus, and iron at certain ages; derangements in the synthesis and translocation of proteins and sugars; phloem necrosis; accumulations of iron at the nodes; weak activity of invertase; and strong amylase activity. Undoubtedly other derangements occur. The total result of these conditions is the cessation of growth of the entire plant, discoloration of the leaves, and dieback of the leaf tips, usually mentioned as the symptoms of potash starvation. The writer is still of the opinion that these derangements are concatenated, as proposed previously (**24**). One of the earliest occurrences in potassium starvation is the increased absorption of other ash constituents, notably phosphorus, calcium, and magnesium, which may enter the plant more readily in the absence of the more mobile potassium. Because enzymes are sensitive to conditions, being readily activated and inactivated, they are soon affected by the lack of potassium. Amylase, not being protected by potassium, is more readily activated by the greater amount of phosphorus, which may cause the accumulation of starch which has been found in some plants. Invertase, peptase, reducase, and probably other enzymes either fail to develop or lose their activity. In the present study

LITERATURE CITED

1. ANDRÉ, G., and DEMOUSSY, E. La répartition du potassium et du sodium dans les végétaux terrestres. Bull. Soc. Chim. Biol. **9**: 861–866. 1927.
2. ————, and ————. Sur la répartition du potassium et du sodium chez les végétaux. Compt. Rend. Acad. Sci. (Paris) **184**: 1501–1503. 1927.
3. BARTHOLOMEW, R. P., and JANSSEN, G. The rate of absorption of potassium by plants and its possible effect upon the amount of potassium remaining in soils from applications of potassium fertilizers. Arkansas Agr. Exp. Sta. Bull. 265. 1931.
4. BROWNE, C. A., JR. Results of investigations at the Sugar Experiment Station Laboratory for 1903. Louisiana Planter and Sugar Mnf. **32**: 47–51. 1904.
5. ————, and BLOUIN, R. E. The chemistry of the sugar cane and its products in Louisiana. Louisana Agr. Exp. Sta. Bull. 91. 1907.
6. BRUNO, A. Hypothèses nouvelles sur le mode d'action de potasse. Rev. Viticulture **69**: 60–62. 1928.
7. BURKSER, E., BRUN, I., and BRONSTEIN, K. Zur Frage der Bioradioaktivität der Pflanzen und der Existenz von Radiumelementen in denselben. Biochem. Ztschr. **181**: 145–148. 1927.
8. CLARK, W. M. The determination of hydrogen ions. 3d ed. Williams and Wilkins Co., Baltimore. 1928.
9. COLIN, H., and BILLON, P. Potasse et betterave. Rev. Gén. Bot. **44**: 5–23. 1932.
10. DOBY, G., and HIBBARD, R. P. Verhalten, insbesondere Ionenaktivierung von Pflanzenenzymen in Abhängigkeit von der Ernährung. I. Mitteilung: Über die Amylase kalihungriger Zuckerrüben. Biochem. Zetschr. 176: 165–177. 1926. II. Mitteilung: Über die Saccharase kalihungriger Zuckerrüben. Biochem. Ztschr. **178**: 139–151. 1926.
11. ————, and ————. Nutrient ions of plants and the ion activation of plant enzymes. Jour. Biol. Chem. **73**: 405–416. 1927.
12. ECKERSON, SOPHIA H. Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. **4**: 119–130. 1932.
13. ENGLIS, D. T., and LUNT, H. A. Effect of the concentration of potassium salts in soil media upon the carbohydrate metabolism of plants. The diastatic activity of the nasturtium. Soil Sci. **20**: 459–463. 1925.

14. FALES, H. A., and NELSON, J. M. The effect of sodium chloride upon the action of invertase. Jour. Amer. Chem. Soc. **37**: 2769–2786. 1915.
15. FALK, K. G. The chemistry of enzyme actions. 2d ed. Chem. Catalogue Co., New York. 1924.
16. FEUILHERADE, L. The presence of starch in the Uba cane. Occurrence and conditions favorable for its presence: Its effect on sugar manufacture and advantages derived from its removal. South African Sug. Jour. **11**: 739, 741. 1927.
17. ————. Starch in Uba juices. Proc. South African Sug. Tech. Assn. pp. 71–74. March, 1929.
18. FOLLETT-SMITH, R. R. The effect of some growth conditions on chemical composition and metabolism in the sugar-cane leaf. Minutes and Proc. of Froghopper Invest. Com. Par. X. Trinidad Gov. Print. Of. Port-of-Spain. pp. 331–347. 1928.
19. GEERLIGS, H. C. P. Cane sugar and its manufacture. 2d rev. ed. Norman Rodger, London. 1924.
20. GOODWIN, T. C., and HANGER, I. C. The ionic nature of amylase. Proc. Soc. Exp. Biol. Med. **23**: 261–263. 1926.
21. GREGORY, F. G., and RICHARDS, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**: 119–161. 1929.
22. GRÜTZNER, P. Über eine neue Methode Pepsinmengen kolorimetrisch zu bestimmen. Pflüger's Arch. ges. Physiol. **8**: 452–459. 1874.
23. HADDON, E. A problem for the factory working Uba juice. Effects of starch and methods of elimination. South African Sug. Jour. **10**: 629, 631. 1926.
24. HARTT, CONSTANCE E. Potassium deficiency in sugar cane. Bot. Gaz. **88**: 229–261. 1929.
25. ————. Studies of the invertase of sugar cane. A preliminary report. Hawaiian Pl. Record **37**: 13–14. 1933.
26. ————. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. Plant Physiol. **9**: 399–452. 1934.
27. JACOB, A. Investigations on the rôle of potassium. (Unpublished). Cited by THOMAS, W. Composition of current and previous season's branch growth in relation to vegetative and reproductive responses in *Pyrus malus* L. Plant Physiol. **7**: 391–445. 1932.
28. JAMES, W. O. Studies of the physiological importance of the mineral elements in plants. I. The relation of potassium to the properties and functions of the leaf. Ann. Bot. **44**: 173–198. 1930.
29. ————. Studies of the physiological importance of the mineral elements in plants. II. Potassium: its distribution, movement,

and relation to growth in the potato. Ann. Bot. **45**: 425–442. 1931.

30. JOHNSTON, E. S., and DORE, W. H. The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. **4**: 31–62. 1929.
31. ————, and HOAGLAND, D. R. Minimum potassium level required by tomato plants grown in water cultures. Soil Sci. **27**: 89–109. 1929.
32. KOSTYTSCHEW, S., and ELIASBERG, P. Über die Form der Kaliumverbindungen in lebenden Pflanzengeweben. Ztschr. physiol. Chem. **111**: 228–235. 1920.
33. LOEB, J. The dynamics of living matter. On the specific difference between the nutritive solutions for plants and animals. pp. 71–77. Macmillan, New York. 1906.
34. LOEW, O. The physiological rôle of mineral nutrients. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bull. 18. 1899.
35. MARTIN, J. P., and MCCLEERY, W. L. Juice quality and cane ripening. 51st Ann. Meeting, H. S. P. A. pp. 411–504. 1932. Advertiser Pub. Co., Honolulu.
36. NEEB, JR., A. P. De enzymen van het suikerriet. Arch. Suikerind. in Nederlandsch-Indie **38**: Deel II: 1001–1003. 1930.
37. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
38. OPARIN, A., and DJATSCHKOW, N. Über die Fermentbildung in reifenden Samen. Biochem. Ztschr. **196**: 289–293. 1928.
39. ————, and KURSSANOW, A. Über die enzymatische Synthese des Rohrzuckers. Biochem. Ztschr. **239**: 1–17. 1931.
40. REED, H. S., and HAAS, A. R. C. Studies on the effects of sodium, potassium, and calcium on young orange trees. California Agr. Exp. Sta. Tech. Paper 11. 1923.
41. RICHARDSON, O. W., and YOUNG, A. F. A. The thermionic work-functions and photoelectric thresholds of the alkali metals. Proc. Roy. Soc. London A **107**: 377–410. 1925.
42. ROBBINS, W. J. Influence of certain salts and nutrient solutions on the secretion of diastase by *Penicillium camembertii*. Amer. Jour. Bot. **3**: 234–260. 1916.
43. SJÖBERG, K. Beiträge zur Kenntnis der Amylase in Pflanzen. I. Über die Bildung und das Verhalten der Amylase in lebenden Pflanzen. Biochem. Ztschr. **133**: 218–293. 1922.

44. Von Stieglitz, C. R. The starch contents of some sugar canes in Queensland. Queensland Soc. Sug. C. Tech., Proc. 3d Ann. Conf. pp. 63–68. 1932.
45. Stoklasa, J. De l'influence de l'uranium et du plomb sur la végétation. Compt. Rend. Acad. Sci. (Paris) **156**: 153–155. 1913.
46. ————. Über die Radioaktivität des Kaliums und ihre Bedeutung in der chlorophyllosen und chlorophyllhaltigen Zelle. Biochem. Ztschr. **108**: 109–139. 1920.
47. ————. Der Mechanismus der physiologischen Wirkung der Radiumemanation und der Radioaktivität des Kaliums auf die biochemischen Vorgänge bei dem Wachstumprozess der Pflanzen. Biochem. Ztschr. **108**: 140–172. 1920.
48. ————, and Pěnkava, J. Über den Chemismus des Zuckerabbaues in der lebenden Pflanzenzelle und den Einfluss der Radioaktivität auf die anaerobe Atmung der Pflanzenorganismen. Ztschr. techn. Biol. **12**: 362–433. 1926.
49. ————, Sebor, J., and Zdobnicky, V. Sur la synthèse des sucres par les émanations radioactives. Compt. Rend. Acad. Sci. (Paris) **156**: 646–648. 1913.
50. Van Den Honert, T. H. Onderzoekingen over de voedingsphysiologie van het suikerriet. Archief Suikerind. in Nederl.-Ind. **40**: Deel III: 1539–1608. 1932.
51. Vines, S. H. The proteases of plants (VI). Ann. Bot. **23**: 1–18. 1909.
52. Viswanath, B. Studies in the chemistry of sugar cane. Agr. Jour. India **14**: 440–475. 1919.
53. Waksman, S. A., and Davison, W. C. Enzymes. Williams and Wilkins Co., Baltimore. 1926.
54. Weller, D. M. Accumulations of starch in the stalks of Natal Uba cane grown on acid soil. Hawaiian Pl. Record **33**: 294–299. 1929.
55. Willstätter, R., and Waldschmidt-Leitz, E. Alkalimetrische Bestimmung von Aminosaüren und Peptiden. Ber. deutsch. chem. Ges. **54**: 2988–2993. 1921.
56. Zwaardemaker, H. On physiological radio-activity. Jour. Physiol. **53**: 273–289. 1920.

GERMINATION OF THE RED SPRUCE

HENRY IVES BALDWIN

(WITH FOURTEEN FIGURES)

Introduction

Since forestry first became a subject for discussion in northeastern America, no problem has received attention longer, nor so frequently, as the question of the natural reproduction of the spruce and fir forests of the northeastern United States and southeastern Canada. Interest was early drawn to it by the pioneer forestry work of GIFFORD PINCHOT and HENRY S. GRAVES in the Adirondacks in 1895–1896, and by the extraordinary growth of the paper and pulp industry based on the utilization of these forests. Until recently this utilization has continued at an ever-increasing rate, faster indeed than progress toward solving the problems of forest replacement.

A great industry and the prosperity of a large section of northern New England and New York are dependent on the perpetuation of spruce and fir in the forest. While hardwoods are being pulped to some extent, and economic surveys point to a shift of some branches of the paper industry, like the lumber industry before it, to the south and Pacific northwest, yet for certain qualities of pulp New England spruce will doubtless always be preferable (8). Without the admixture of spruce and fir, the forests of northern hardwoods would have far less economic value and would lose much of their charm for the tourist, and the wooded mountain sides would be less effective regulators of stream flow.

In the 35 years since spruce reproduction was first studied systematically in this country, knowledge of the factors affecting it has increased slowly. Our understanding of the spruce-fir forest environment is vastly greater, but little has been published on the germination of red spruce (*Picea rubra* Link.). A study of natural reproduction by seed should start with consideration of the seed. With few exceptions this has not been done. Without a thorough knowledge of the seeding habits of the species and of the behavior of the seed, as influenced by various environmental factors, however, a true understanding of the phenomena of establishment cannot be acquired. Only when the most favorable conditions for germination are known can the silviculturist plan cutting methods best calculated to secure these conditions.

The investigations reported here are studies of seed behavior. They do not attempt to go beyond the germination process itself. It must be conceded, however, that the success of spruce reproduction is often as much or more dependent on factors affecting survival during the critical first months

following germination, or even during the first few years, than upon germination itself. It is planned to deal with the survival and establishment of seedlings more fully in a later publication.

Historical survey

The literature on seed germination is so extensive that the present review is restricted chiefly to that concerning red spruce. KORSTIAN (30) has recently reviewed the literature on seeds of broad-leaved trees; HAASIS (19) has dealt with the special subject of temperature coefficients of the germination of coniferous seeds; and JOSEPH (24) and BARTON (5, 6) have pointed out the importance of after-ripening in many arborescent species. Finally, TOUMEY and STEVENS (58), SCHMIDT (49), and TOUMEY and KORSTIAN (56) have brought together much of the existent information on forest tree seed.

Careful accounts of the germination of red spruce seeds under natural or artificial conditions are comparatively scarce, and for the most part, recent. The first observations appear to have been made in America. Many papers might be cited, few of which describe germination itself. PINCHOT (41) published the first silvicultural study of red spruce. He states that the seeds, "where there is light enough, germinate and grow easily on deep spruce or pine duff, and on heavy beds of moss, . . . a heavy matting of leaf litter . . . is less thoroughly adapted to their requirements." GRAVES (18) makes a similar statement regarding germination. The ideas about the importance of light which prevailed at the beginning of the century have gradually changed as the decisive rôle of soil moisture has become better appreciated. CHITTENDEN (13) believed red spruce seeds do not germinate as readily as those of balsam fir (*Abies balsamea* (L.) Mill.) and are more exacting in regard to seed bed. He recognized, however, that no special light intensity is required for germination. MURPHY (35) compared the establishment of the two species in detail. He stressed the fall germination of spruce as a factor placing it at a disadvantage with fir. During the autumn he believed the soil moisture and other factors to be less favorable for the germination of spruce in the open than in the forest. Assuming that the chief seed distribution in the open occurred later in the winter, he concluded that the percentage of spruce seed which would lie over for spring germination would be greater in the open than in the forest. In a later publication (36) he emphasized the importance of soil moisture in controlling germination. The heavy mat of hardwood leaves under hardwood stands often prevents the seed from reaching moist soil, or, germination having taken place, the seedlings are unable to emerge, and succumb to mold under the poorly aerated mulch. A similar explanation was given by MURPHY and ZON (37).

MOORE (32) experimented to determine whether red spruce seeds remained alive when stored in the duff. He transferred flats of undisturbed humus, dug up in the dense forest, to an opening and watered them. Some seeds were sown in the corners of the flats. The results were inconclusive, since it was uncertain whether the seeds which germinated were ones stored naturally in the duff, or artificially sown. In another case (33) he grew seedlings of spruce and fir in flats filled with various soils. The spruce grew best in mild humus, but withstood high acidity better than fir. In a further study he states: "Germination occurs directly in the humus, contact with the mineral soil seeming unnecessary." He considered hardwood leaves unfavorable, not only hindering penetration of the spruce roots, but also preventing the shoots from coming to the surface. He thought it probable that spruce seedlings germinated and grew between the leaves a certain distance, but were unable to push through them. WESTVELD (61, 62) has recently carried out experiments on seed-bed conditions for red spruce. Seed was sown in the fall on the undisturbed litter and on plots cleared of litter. On June 11 the following spring there was an abundance of spruce seedlings on the cleared plots, but none on the undisturbed litter. By fall, however, a number of spruce seedlings had appeared on the undisturbed plots as well as an increase of 75 per cent. on the cleared plots. This would seem to indicate that even after favorable conditions for after-ripening during the winter, there is considerable delay in the spring germination on litter. Judging from the promptness with which germination occurs under optimum conditions with dry or after-ripened seed, it seems likely that these results were due to fluctuations in soil moisture, or that additional seed was shed during the summer.

There is considerable scattered information on the size, quality, and germination of red spruce seed as the result of laboratory tests. Red spruce cones give about the same yield of seed as many other conifers. TOUMEY and KORSTIAN (56) give the average as 1.03–1.04 lb. clean seed per bushel of cones. STETSON (50) found an average of 8843 seeds per oz., or 3.06 gm. per 1000 seeds. RAFN (42) gives 3.6 gm. per 1000 (only one sample tested). TOUMEY (53) gives the average number of clean seeds per pound as 131,400, or 3.16 gm. per 1000. JACOBSEN (23) gives the following weights per 1000 (in gm.):

Highest 3.4	Lowest 2.8	Average 3.1

TOUMEY and KORSTIAN (56) quote TILLOTSON (52) as finding 130,000 seeds per pound, and BUELL (11) 139,200 for North Carolina seed. There would thus appear to be a possibility of some difference in weight of seed in different parts of the tree's range. Ordinarily the heaviest seeds are borne in the more southerly parts of the range.

The same investigators give some scanty information on germination. SUDWORTH (51) gives the germination of fresh seed as 60–70 per cent.; STETSON (50) obtained 25 per cent. with 1909 seed and FAHRENBACH (17) but 6 per cent with 1910 seed. RAFN (42) reported 31.7 per cent. in 30 days from one lot, and JACOBSEN (23) lists the average of three lots as:

High 76 per cent. Low 68 per cent. Average 71 per cent.

Her tests were continued 28 days on the JACOBSEN apparatus at 16–28° C. alternating temperature. TOUMEY (53) and TOUMEY and KORSTIAN (56) give 54 per cent. as the average in soil tests. MURPHY (36) states that "50–90 per cent. of the seed are perfect, and of these 60–80 per cent germinate."

Few exact studies of the germination requirements for red spruce appear to have been made. Forestry and ecological literature abounds with general statements about the most favorable seed-bed, and with theories of the requirements for acidity or light. Observations of fall germination suggest that prolonged after-ripening is not required. Moisture seems to have been found the outstanding factor influencing germination.

Investigation

DESCRIPTION OF RED SPRUCE SEED

Mature red spruce seeds are very dark brown, about 3 mm. long, with short broad wings rounded above the middle. The testa, or coat, consists of an outer crustaceous layer, dark brown and occasionally mottled or streaked, and an inner membraceous layer, pale chestnut brown and lustrous. The pale yellow embryo is axile in a large fleshy endosperm. The number of cotyledons varies from four to seven. While slightly larger, the seeds of red spruce are often difficult to distinguish from those of black spruce (*Picea mariana* (Mill.) B.S.P.) and from white spruce (*Picea glauca* (Moench.) Voss) when the wings are lacking. Illustrations of the seeds are given by SARGENT (47).

FACTORS AFFECTING GERMINATION

The phenomenon known as germination consists of a renewal of activity of the embryo, much as the vegetative parts of a tree begin to grow again after a resting period. The criteria by which one judges whether germination has taken place are largely arbitrary. BRANDTS (9) has suggested that germination be considered to take place when the first new cell divisions occur in the formative region of the root. Generally, however, emergence of the radicle or hypocotyl, so that it projects beyond the edge of the testa, is accepted as a criterion of germination. In soil tests emergence of the seedling above ground is so accepted.

In the developing embryo, as in the branch bud, both external and internal factors are operative in causing renewed activity. The internal fac-

tors are those inherent in the seed, or such causes as dormancy, size and weight of seed, age, degree of maturity, and hereditary characteristics. CROCKER (14, 15) has classified the causes of dormancy, and shown that in some species delayed germination is occasioned by a dormant embryo; in others, by inhibitory seed coats or combinations of these factors. In seeds capable of immediate germination, external factors such as temperature, moisture supply, and oxygen supply are the chief controls of germination. Light is rarely of importance in the germination of tree seeds, and the greatest influence of solar radiation is in its effect on temperature and soil moisture, as shown by BOERKER (7). Soil acidity and carbon dioxide concentration no doubt affect germination in nature, either directly or indirectly. Of the possible rôle of microorganisms little is known. . .

CHARACTER AND SCOPE OF INVESTIGATIONS

The investigations undertaken consisted partly of field and partly of laboratory experiments.

I. FIELD EXPERIMENTS.—The field work was begun as part of an investigative project for the Brown Company on the Cupsuptic Experimental Area in Adamstown, Maine, in the fall of 1924. Experiments were later continued and augmented by plots established on the Dummer Experimental Area, Dummer, New Hampshire, in 1925, and on the Jerico Forest, near Berlin, New Hampshire, in 1926. These experiments were planned and executed with the following objectives:

1. To determine the rate of germination and survival of spruce under different soil conditions. A large number of small seed plots were prepared and sown with spruce seed. Some were covered with spruce branches, some with wire cages to exclude birds and animals, and some were left open and unprotected. Germination and survival data were obtained by counts at intervals. A brief summary of these results is given only in so far as they touch upon the present problem.

2. To determine the rate of establishment of natural spruce reproduction on quadrats subjected to special treatment:

(a) Exposure of quadrats denuded of humus, herbaceous and woody shrubs, or by combinations of all these treatments.

(b) Comparison of trenched and untrenched quadrats to demonstrate the influence of competition for soil moisture and nutrients.

3. To determine the length of retention of vitality of seed stored in the duff by:

(a) Testing seeds taken from duff samples under various conditions of site and forest type.

(b) Testing seeds stored under cages in insect-proof copper wire boxes buried in the duff on various sites.

II. LABORATORY EXPERIMENTS.—The laboratory work comprised the following experiments on red spruce germination under controlled conditions, so far as practical to attain, in order to determine the effect of the various factors on germination. Experiments were conducted on:

1. The moisture and ash content of the seeds used in the experiments.
2. The average germinative energy of seeds stored for different periods.
3. Germination at different constant temperatures.
4. Moisture supply: relation of water absorption to germination.
5. Germination in light and darkness.
6. After-ripening treatment.
7. Oxygen supply: germination under water with and without aeration.
8. Germination in different hydrogen-ion concentrations.

MATERIAL

Red spruce seed collected in northern New Hampshire was used in these experiments. It was extracted at approximately 50° C., winged and cleaned dry at the Brown Co. Seed Extracting Plant at Berlin, New Hampshire. In the field work seed collected in 1924 and 1925 was used; for the laboratory experiments seed of the 1927 and 1928 commerical collections was used. It was stored air-dry in sealed tins at approximately 15° C. until June, 1929, in the case of the 1927 seed, and October, 1929, in the case of the 1928 seed. From then until placed to germinate it was kept in tightly corked glass bottles at room temperature, or in a cellar at approximately 15° C. In some of the last tests similar seed from the 1929 crop was used.

The 1927 lot was found to weigh 3.222 gm. per 1000 filled seeds. Empty seed weighed 37 per cent. as much as filled seed. The 1928 lot contained 7.8 per cent. moisture, and the ash was 4.7 per cent. of the dry weight. In the laboratory work 70,000 counted seeds were used. Most experiments were run with eight duplicate samples of 100 seeds each, for each variable condition.

Experimental methods and results[1]

I. FIELD EXPERIMENTS

1. GERMINATION AND SURVIVAL ON SEED PLOTS.—In connection with direct seeding experiments, observations were made on germination and survival under various conditions of soil and cover. The experiments were made in 1925 at Cupsuptic Lake, Maine, and in 1926, 1928, and 1929 at Berlin, New Hampshire.

Plots about 30 cm. square were prepared with a grub hoe by stripping off the sod or upper layers of humus. Seeds were sown and gently pressed

[1] Source tables are omitted on account of lack of space. They may be consulted in bound copies of the original manuscript on file in the Yale University Library.

into the soil, or at times lightly covered. In many cases 100 seeds were sown; in others, less. At the time of sowing, notes were taken on the character of the soil in each plot. Some plots were protected with spruce and fir branches, and about 60 with small wire cages to prevent molestation by birds and animals. Alternate plots were left unprotected. Each plot was designated by a small numbered stake to make relocation certain. Plots were examined and seedlings counted once or twice a year. In the different experiments about 1000 plots were studied.

In all cases more seedlings were obtained under the wire cages, owing possibly to conservation of soil moisture by the sides of the cage as much as to protection from birds. In the later experiments about 100 per cent. more seedlings appeared in the protected plots than in the unprotected ones. Sand or mineral soil proved better than humus or rotted wood as a seed-bed, confirming previous conclusions by many observers of red spruce as well as those of BARR (4). Germination and survival were higher when the seed spots were protected by spruce boughs than when uncovered and exposed. These shaded the surface soil from direct sunlight and prevented loss of moisture, especially after the needles dropped off and formed a mat on the soil. It has been repeatedly shown that freshly germinated tree seedlings can be killed by high surface soil temperature. The best seed-bed as indicated by these experiments would be found where moisture is available at all times, and where there is adequate protection against surface temperatures above 50° C. (TOUMEY and NEETHLING, 57.)

2. NATURAL SEEDING AND ESTABLISHMENT AS RELATED TO SOIL CONDITIONS. —The results of natural seeding on denuded quadrats were rather disappointing, since very few seedlings appeared. This was attributed partly to the poor seed supply where the plots were located, and partly to the destruction of seed by various agencies.

In the fall of 1925 a plot two milacres in area under a fully stocked stand of mature red spruce in Dummer, New Hampshire, was trenched, severing all roots entering the plot to a depth of 0.5 m. Trenching was repeated in 1926 and 1928. An adjacent untrenched plot was established as a control. Spruce and fir seedlings and other vegetation on the plots were mapped and photographed at intervals. New seedlings which germinated were designated by colored toothpicks. At the start the vegetation on the two plots was practically identical, but after two years the tree seedlings, herbs, and mosses were noticeably larger and more abundant on the trenched plot. It is not known whether each plot received the same quantity of seed, but germination and survival were much greater on the trenched plot, owing chiefly to the more abundant soil moisture. Mention is made of these plots by TOUMEY and KIENHOLZ (55).

3. RETENTION OF VIABILITY BY SEED STORED IN DUFF OF FOREST FLOOR.—The rapid establishment of seedlings of some trees on recent cuttings or burns where there are no seed trees in the vicinity or when there has been no seed crop recently has led foresters to conclude that such reproduction arose from seed stored in the duff. HOFMANN reached this conclusion, since largely disproved in the case of Douglas fir by the investigations of ISAAC (63) and HAIG (20). CARY (12) states that seed stored in the duff must be relied upon for reproduction of Sitka spruce (*Picea sitchensis* (Bongard) Carrière) in Alaska. The possibility of spruce seed being stored in the duff in eastern forests has been suggested by MULLOY (34) and others, but few definite experiments have been made.

Two methods were used for investigating viability of seed stored in the duff. (a) Samples of duff were analyzed and germination tests applied to seed recovered. (b) Seeds of known germinative capacity were sown un-

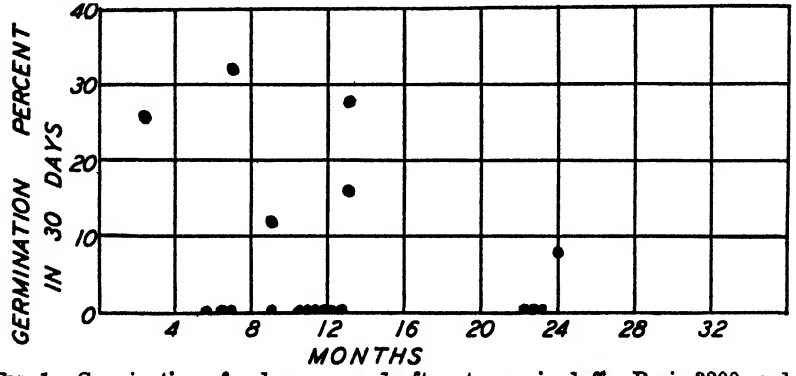


FIG. 1. Germination of red spruce seed after storage in duff. Basis 3200 seeds.

der the litter, or inclosed (thoroughly mixed with duff) in fine-mesh wire cages and buried in the duff. After remaining buried a known length of time, the cages were dug up and the seeds recovered and tested for germination, in comparison with seeds of the same lot stored in the laboratory. The first method suffers from several objections: it is impossible to determine the age of the seed recovered; it may be freshly fallen; its original germinative capacity and hence its deterioration in storage are unknown. Results of duff sampling depend very largely upon the location, the proximity of seed trees, and the density of the local rodent and bird population, which might consume a considerable amount of the best seed. The second method overcomes some of the disadvantages of the first, while open to the objection that the method of storage is slightly less natural.

(a) *Analysis of duff samples.*—The first samples were taken at Cupsuptic Lake in the summer of 1925 before the seed of the year was ripe. Later, samples were taken in the autumns of 1925 and 1926 in Dummer,

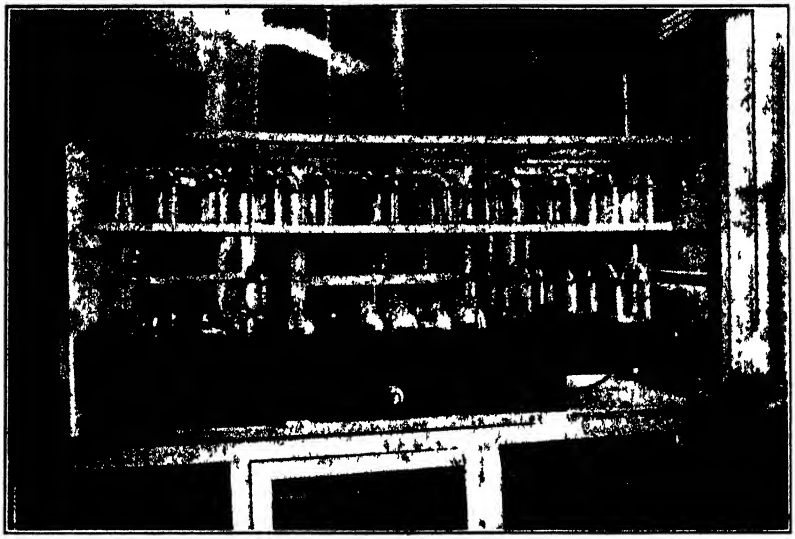


FIG. 2. JACOBSEN type germinator with front of case removed, showing also individual JACOBSEN germinators

New Hampshire An area 30 cm square was laid out and a section 10 cm. deep was cut and lifted out with a large machete It was brought into the laboratory and dried for several weeks at room temperature in a tray

FIG. 3. Individual JACOBSEN germinators.

TABLE I

ANALYSIS OF DUFF SAMPLES, SIZE 30 × 30 × 10 CM.

SAMPLE NO.	DATE LIFTED	DESCRIPTION OF DUFF	LOCATION	FOREST TYPE	CROWN DENSITY	EXPOSURE	NO. OF SOUND SPRUCE AND BALSAM SEEDS RECOVERED	GERMINATION
								%
1	Sept. 8 1925	*Hylocomium* moss, bottom dead moss, and rotted wood	Plot S-3 Cupsuptic Lake, Me.	Pure spruce knoll, cut clean 1924–5	0.0	W	174	0
2	Oct. 16 1925	Spruce needles in all stages of decay	Plot B-25 Dummer, N. H.	75-yr. pure spruce	1.0	S	460	0
3	Nov. 17 1925	Beech and sugar maple leaves	Near R-35 Dummer, N. H.	Old growth, hardwood, 100 m. east of spruce	0.8	E	17	0
4	Oct. 14 1926	Spruce needles	Plot B-25 Dummer, N. H.	75-yr. pure spruce, edge of stand	0.5	W	300 +	0
5	"	Spruce needles	Plot B-25 Dummer, N. H.	75-yr. pure spruce, center of stand	1.0	S	38	0
6	"	Beech and maple leaves	Near R-35 Dummer, N. H.	Culled virgin hardwood	0.7	E	4	0

screened against rodents and insects. When thoroughly dry, the sample was rubbed through wire screens of progressively finer mesh until the seeds were caught. The seeds thus obtained were counted and tested for germination. Duff samples from locations where seeds had been shed recently, as the cut-over sites at Cupsuptic Lake, contained abundant seeds, as shown in table I. All attempts to cause these seeds to germinate failed, however. Six samples of these seeds were submitted to the Boyce Thompson Institute for Plant Research for testing. From these only one seed germinated. Most of the others seemed to have been attacked by fungi (table II).

TABLE II

TESTS OF SPRUCE SEED RECOVERED FROM DUFF SAMPLES*

No. of sample	No. of seeds	No. without embryo	No. with embryo	No. lighter than water (of seeds with embryo)	No. heavier than water	No. germinated
1	76	54	22	22	—	—
2	3	1	2	2	—	1
3	14	9	5	5	—	—
4	195	140	55	53	2	—
5	36	31	5	5	—	—
6	74	73	1	1	—	—

* Tests made by Boyce Thompson Institute, January, 1926. All seeds except no. 2 were heavily attacked with mold from the start. All seeds had the seed coat cut open in one place to examine for presence or absence of embryo. They were sterilized with 0.25 per cent. uspulun for 5 minutes before being placed to germinate.

(b) *Burying seed samples.*—In burying seed for subsequent digging up and examination, use was first made of frames 1 × 1.5 m. covered by 1 cm. galvanized wire netting. These were pressed firmly down over the spots where the seeds were buried, and fastened rigidly to the ground by stakes nailed to the sides. Normal growth of ground vegetation was not interrupted. The seeds were buried in pockets about 5 cm. beneath the surface, the duff being lifted with a knife for the purpose. About 500 seeds were placed in each pocket, and marked with a small stake. About 20 lots of seed were accommodated in each cage. The seed used was collected and extracted in the fall of 1925. Wings remained attached to the seeds. The cages were established in three different forest types in the fall of 1925. In 1926 small copper gauze boxes containing a known number of seeds mixed with the duff were buried. With these it was possible to recover every seed buried. Whenever it was possible to visit the stations, temperatures in the duff were read from current thermometers permanently hung in wooden tubes.

TABLE III

GERMINATION OF RED SPRUCE SEED STORED IN THE DUFF, 100 SEEDS PER SAMPLE. TESTS MADE ON THE JACOBSEN GERMINATOR AT 24° C.

SAMPLE NO.	CHARACTER OF DUFF WHERE BURIED	TIME BURIED	PERCENTAGE GERMINATED AFTER			REMAINING SOUND SEED	GERMINATION CAPACITY
			30 DAYS	60 DAYS	90 DAYS		
		months	%	%	%	%	%
33	Spruce raw humus	2.5	26	26	26	2	28
37	" " "	3	0	0	0	0	0
34	" " "	5.5	0	0	0	0	0
7	" " "	7	32	42	44	0	44
8	Beech-maple leaves	6.5	0	0	0	0	0
19	" " "	11	0	0	0	0	0
13	Balsam-birch-aspen litter	11.5	0	0	0	0	0
15	Spruce raw humus	11.5	2	3	3	0	3
16	" " "	11.5	0	0	0	1	1
18	Balsam-birch aspen litter	11.5	0	0	0	2	2
35	Spruce raw humus	12	0	0	0	0	0
36	" " "	12	0	0	0	0	0
28	Balsam-birch-aspen litter	13	16	16	16	5	21
29	Balsam-birch-aspen litter	13	28	28	28	2	30
21	Spruce raw humus	23	0	0	0	2	2
22	Balsam-birch-aspen litter	23	0	0	0	46	46
23	Beech-maple leaves	23	0	0	0	32	32
39	Balsam-birch-aspen litter	24	8	8	8	8	16
25	Spruce raw humus	33	0	0	0	0	0
20	" " "	33	0	0	0	1	1
26	Beech-maple leaves	33	0	0	0	9	9
27	" " "	33	0	0	0	21	21
	Control samples of same lot of seed, stored in laboratory, and germinated at same time as above						
1	Dry, in corked bottles	0	58	60	60	9	69
3	Dry, in corked bottles	3	43	48	48	7	55
11	Dry, in corked bottles	6.5	12	48	53	2	55
14	Dry, in corked bottles	11.5	43	44	44	1	45
17	Dry, in corked bottles	11.5	28	37	37	0	37
24	Dry, in corked bottles	23	63	63	63	23	86

Better success attended these experiments. Samples removed from the duff were tested in the JACOBSEN germinator simultaneously with controls of the same lot of seed kept in air-tight containers in the laboratory. The results are shown in table III. The latter lot of seed lost little germinative energy during 25 months, while that stored in the duff decreased 50 per cent. in eight months and after 11.5 months gave only 1.7 per cent. as great germination as the control. In one case 8 per cent. germination was obtained after two years, but this was exceptional. In many cases seeds were found which had germinated in the duff. Where moisture was adequate, conditions were favorable for germination throughout the growing season, since the temperature was sufficiently high (table V), as will be shown later. Seeds which had after-ripened by storage during the winter were able to germinate at still lower temperatures. This tendency to germinate as soon as conditions became favorable probably explains why so few seeds remained dormant over summer. HAIG (20) has recently reported the same phenomenon in western conifers.

SUMMARY OF FIELD EXPERIMENTS

From seed plot trials the importance of moisture conservation in the surface soil and of moisture availability was apparent. Germination was most successful on sand or mineral soil and under cover of spruce branches or needles. Similar results were observed on extensive trials of direct seeding, and on plots depending on natural seeding. The removal of root competition by trenching was followed by more abundant germination. Some spruce seeds remained viable in the duff about one year, but had deteriorated greatly in germinative capacity by that time.

II. LABORATORY EXPERIMENTS

1. AVERAGE COURSE OF GERMINATION.—Seventy-six lots of red spruce seed tested during the years 1927–30 gave an average germination of 77 per cent. in 30 days for fresh seed and 62 per cent. in 30 days for 3-year-old seed. Tests were made in the JACOBSEN apparatus at a constant temperature of 24° C. Figure 4 shows smoothed curves of the normal course of germination after varying periods of dry storage in corked bottles at room temperature. Fresh seed germinated more promptly and gave higher energy values at 30 days than seed which had been stored longer. In the stored seed, that 2–3 years old germinated slightly more promptly than that 1–2 years old. Final values were higher for the latter, however. The germinative energy in 30 days was found to decrease about 10 per cent. per year for the first three years. With increasing age there ensued a progressive decrease in germinative energy, as well as in the final germination percentage and germinative capacity. Frequently the embryo and endosperm became

TABLE IV

AVERAGE VIABILITY OF SEED AFTER STORAGE IN AIR-TIGHT CONTAINERS AT ROOM TEMPERATURE
AVERAGE COURSE OF GERMINATION FOR 1927–1930,
JACOBSEN GERMINATOR. TEMPERATURE 24° C. CONSTANT

	COURSE OF GERMINATION AVERAGE PER CENT. GERMINATED AFTER						GERMINATIVE ENERGY IN 30 DAYS			REMAINING SOUND SEED AV.	GERMINATIVE CAPACITY AV.	BASIS NO. OF TESTS OF 100 SEEDS EACH
	5 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS	HIGH	AV.	LOW			
	%	%	%	%	%	%	%	%	%	%	%	
Seed stored less than 1 year ...	13	66.5	76.0	76.7	77.2	77.2	87	77.2	66	7.0	84.2	4
Seed stored 1–2 years	0.4	40.7	59.2	65.8	67.0	68.0	91	68.0	27	7.8	75.8	47
Seed stored 2–3 years	6.3	48.7	54.4	58.6	60.3	62.0	88	62.0	30	9.2	71.2	25

shriveled and nearly disappeared in old seed, giving rise to an apparent increase in the percentage of empty seed. By suitable recleaning, these blind seeds can be eliminated, thus restoring to a lot of seed much of its original germinative value.

2. GERMINATION AT DIFFERENT CONSTANT TEMPERATURES.—Temperature affects seed in many ways. Summer heat affects the ripening of the seed, the heat (through its drying effect) influencing extraction from the cone, whether by natural or artificial means. Temperature is important while the seed is in dry storage or in the process of after-ripening in the soil, and finally, it is frequently a limiting factor in germination.

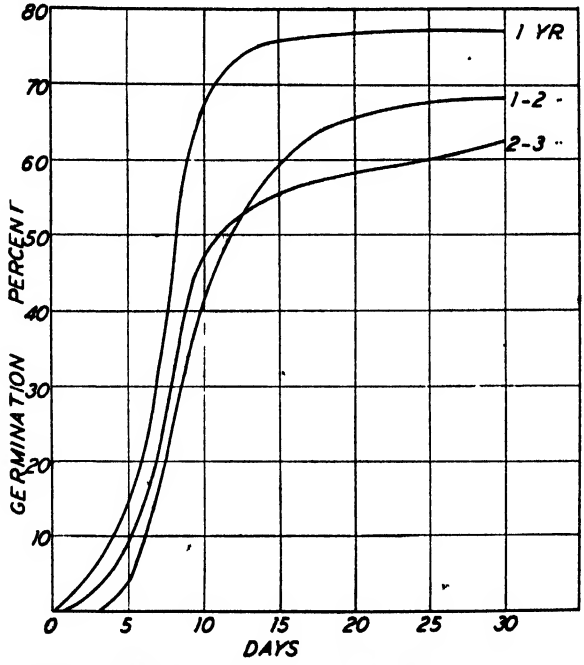


FIG. 4. Average curves of germination of red spruce seed grouped by duration of storage. JACOBSEN germinator at 24° C. constant. Basis one year, 800 seeds; 1–2 years, 9400 seeds; 2–3 years, 5000 seeds.

The following experiments were made to determine the temperature requirements for germination of red spruce. They were begun in March, 1930, and extended over six months.

Seeds of the 1928 crop were used, 800 for each temperature at which tests were made. They were counted out from the air-dry seed and set to germinate without treatment, except in a few cases. Containers for the seeds were prepared as follows: Petri dishes 10 cm. in diameter were filled to a depth of 1 cm. with pure quartz sand. These were covered with filter

papers fitting the dishes exactly. The dishes were then saturated with distilled water and the excess drained off by tipping the dish. Then 100 seeds were laid on the filter paper and the dishes covered. Moisture was renewed at the time of counting the sprouted seeds.

The seeds were germinated in a specially constructed series of Freas chambers at the following temperatures: 10°, 20°, 24°, 28°, 32°, and 38° C. The petri dishes were placed in stacks of four each, and at each counting the positions of the dishes in the stack were changed. The tests were examined daily at the same hour, and the sprouted seeds removed, usually at two-day intervals. The seeds were kept in darkness except for 2–3 minutes at the time of each examination. Uniform temperatures were maintained

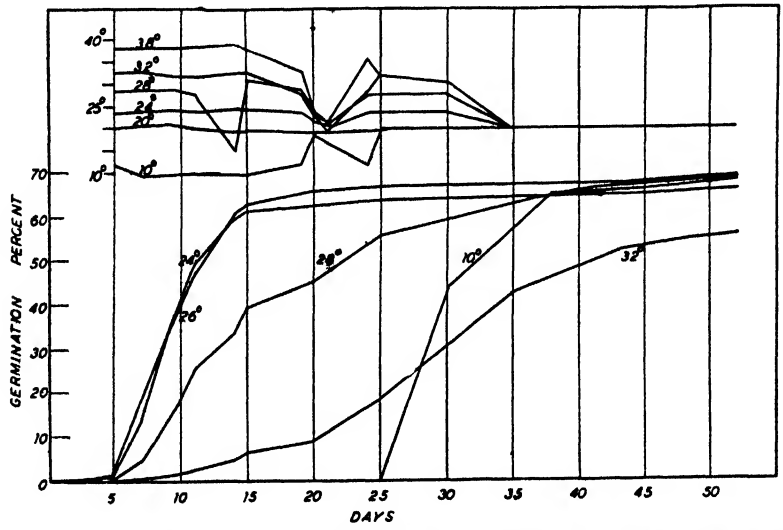


FIG. 5. Germination of red spruce at different temperatures, first series. Basis 800 seeds each curve.

except for the following fluctuations: On the 11th day the 28° chamber suffered a sharp drop in temperature, and after the 20th day all chambers were brought to 20° for four days. At the expiration of this time the original temperatures were resumed and maintained until the 30th day. On the 25th day, no germination having occurred in the samples stored at 10°, the samples were removed from the ovens to a cellar where they remained at approximately 20° C. in diffuse light until the tests were discontinued. The fluctuations in temperature are shown graphically in figure 5.

In May, 1930, a second series of tests was set up in the same ovens, using samples from the same lot of seed. The same germinating medium was used as in the first series, but in this case the petri dishes, complete

with sand, filter paper, and water, were autoclaved for 15 minutes at 20 lb. steam pressure and cooled to room temperature before inserting the seed. In this experiment all samples were examined, and sprouted seeds removed and recorded daily at the same hour. Temperatures in the chambers were read to 0.1° C. at each examination. During this second series two changes in the uniformity of the temperature occurred, one for six hours on the fourth day, and again for an equal period on the eighth day. In addition, the temperature in the 26° chamber rose to 32° C. for a period of unknown length on the thirteenth day. The temperatures maintained (except as just noted) were 10°, 15°, 18°, 20°, 24°, and 26° C.

The summarized results are shown in table V and figures 5–7. In the first series the samples at temperatures of 20° and 24° were the only ones displaying a normal course of germination. No germination whatever occurred at 10° or at 38° C. At 28° C. the inhibiting effect of high temperature was already very marked, and satisfactory germination began only after the drop in temperature on the eleventh day already mentioned. When the ovens were shut down and the temperature in this compartment fell, germination was completed normally. The same was also true of the samples at 32° C. The seeds exposed to 38° C. were probably killed after a short period, since a subsequent drop in temperature did not induce germination. In the case of the low temperatures, 10° C. was below the minimum for germination. In neither of the series did germination occur at 10° C. during the period of the tests (20–30 days). In the second series, four samples which had been after-ripened in moist peat at 5°–7° for two months were incubated at 10° C., but no germinations occurred. Upon completion of the tests, these samples, as well as the others from the 10° C. oven, were placed in a refrigerator and kept continuously at 5°–7° C. for 133 days. During this time 1–2 per cent. germinated, indicating that, as a result of prolonged after-ripening, the minimum temperature for germination had become lower. These samples were then placed in the ground under a spruce stand in New Hampshire where the temperature varied from 0° to 5° C. They were allowed to remain there one month, during which 4 per cent. germinated. Then extreme weather necessitated their removal to a cool cellar. During the next month in the cellar at approximately 8° C. about 20 per cent. more germinated. The tests were then discontinued.

In October, 1930, experiments were made to determine whether seeds would germinate out-of-doors under the prevailing temperatures. Owing to the severe drought prevalent at the time, it was arranged to keep the seed-bed uniformly moist, while otherwise simulating natural conditions as closely as possible. The following apparatus was used: copper cylinders 10 cm. in diameter and 15 cm. high were constructed, having bottoms of very fine

TABLE V

SUMMARY OF GERMINATION OF RED SPRUCE AT DIFFERENT CONSTANT TEMPERATURES*

TEMPERATURE	SERIES	COURSE OF GERMINATION — DAYS																						
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	35	38	43	48	52
°C.		%																						
10	I																	0.1	43.6		65.0			68.0
	II	(1.7% after 133 days, 5.5% after 160 days, 26.2% after 190 days)																						
15	II					0.8	2	5.4	11.4	15.9	24.9	26.9	30.8	32.5	33.6	36.6	38.6							
18	II			1.2	7.1	17.6	28.4	38.6	48.1	55.4	60.0	61.7	64.1	65.7	66.4	68.1	68.9							
20	I			12.5		31.9		45.9			60.0	62.2					65.0	66.1	66.4	66.6		67.2	68.1	68.9
	II		0.4	13.0	27.6	37.5	45.6	53.2	56.4	59.1	61.8	62.6	63.5	63.7	64.1	65.0	65.2							
24	I	0.7		16.9		32.6		48.6			59.2	61.2					62.7	63.7	64.0	64.2		65.1	65.2	65.9
	II	2.8	6.5	24.5	36.2	44.2	52.4	57.4	60.5	62.7	64.0	64.6	65.0	65.2	66.0	66.2	67.0							
26	II	3.0	11.5	28.5	38.4	50.2	58.4	61.5	62.8	65.0	66.0	66.8	67.0	67.4	67.9	68.5	69.1							
28	I	0.4		4.6		12.7		25.1			33.2	39.1					44.5	55.7	59.2	62.7		66.1	68.1	68.5
32	I			0.4		1.1		2.2			4.7	6.6				8.7	9.0	18.2	30.4	42.5		52.0	54.5	56.2
38	I	No germination																						

* Basis 800 seeds each temperature and series; total 9600 seed.

TABLE V—(*Continued*)*

Temperature	Series	Remaining seed			Germination values		Variation between samples	
		Good	Bad	Empty	Real germination in 20 days	Real germinative capacity	Average deviation	Standard deviation
°C.								
10	I			8.5	42.7	83.5	5.5	6.9
	II			3.1	70.8	87.2	4.4	4.7
15	II	43.7	8.0				3.2	4.3
18	II	17.4	9.5	7.2	71.2	87.4	5.7	6.8
20	I	15.0					7.2	9.8
	II	12.0	7.7	5.0	71.8	90.5	2.4	3.2
24	I			2.4	72.5	88.4	2.8	3.6
	II	17.5	8.6				3.2	4.7
26	II	15.2	10.8				1.7	2.6
28	I							
32	I							
38	I							

* Basis 800 seeds each temperature and series; total 9600 seed.

(80-mesh) copper gauze. They were mounted on 1.5-cm. legs. These cylinders were filled to within 2 cm. of their tops with fine sand, previously cleaned by washing with 20 per cent. HCl and distilled water. The cylinders were then placed in an enameled pan filled with water to a point 1 cm. above their wire bottoms. This level was subsequently maintained automatically by a Mariotte flask rigidly attached to the pan. The remainder of the water surface in the pan was covered with glass strips held in place with adhesive plaster and heavy paraffined cardboard to prevent evaporation and to exclude dirt. The whole was then given a coat of waterproof lacquer. The apparatus was buried in the ground so that only the tops of the cylinders protruded. The sand surface within the cylinders was adjusted level with the surface of the ground (fig. 8). Two such complete units were established, containing three cylinders each, one in an opening and one under dense shade in a 20–40 year-old stand of old field spruce near Berlin, N. H. The ground surface in the open was covered with a heavy sod and spruce seedlings were abundant. Under the stand there was no ground flora, but a thick mat of spruce needles covered the surface. One hundred seeds of the 1928 crop were sown in each cylinder and gently pressed into contact with the sand. Maximum and minimum thermometers with bent stems for measuring surface soil temperatures (31) were kept inserted in the sand surface and read daily. When temperatures continuously below freezing ensued in mid-November, no germination had yet occurred in any of the cylinders, and both units were removed to the laboratory. Here germination began promptly and continued for nearly a month from seed which had become deeply buried. Owing probably to the poor quality of the 1930 crop, some seeds collected from trees on the site of the experiment and sown along with the standard seed failed to germinate. Probably the experiment should have been started earlier in order to encounter conditions suitable for fall germination. Seed shed early in September may thus in some years meet with temperatures favorable for germination. Periods of warm weather also occur occasionally in October. From several years' observation, however, only exceptionally have fall-germinated red spruce seeds been seen.

Summary of effect of temperature

The effective range of temperature within which germination occurs normally was found from the preceding experiments to be approximately 15°–30° C. The optimum temperature apparently lay between 24° and 28° C. For after-ripened seed the minimum was lower. Imbibed seeds exposed to temperatures of 32° C. and higher seemed to incur permanent injury. On the other hand, incubation at temperatures below the effective range resulted in very rapid germination when the temperature was raised to a point within the optimum range.

3. RATE OF MOISTURE ABSORPTION.—Red spruce seeds belong to the group with moderately high germinative energy. They germinate in a few days after the conditions become favorable. PETTIS (40) gives the time necessary for red spruce seeds to germinate in the nursery as 12 days. The shortest time between placing dry seeds in the germinating medium and the starting of germination observed by the writer was four days; seven days is perhaps the average. Seeds buried in soil do not appear above ground until

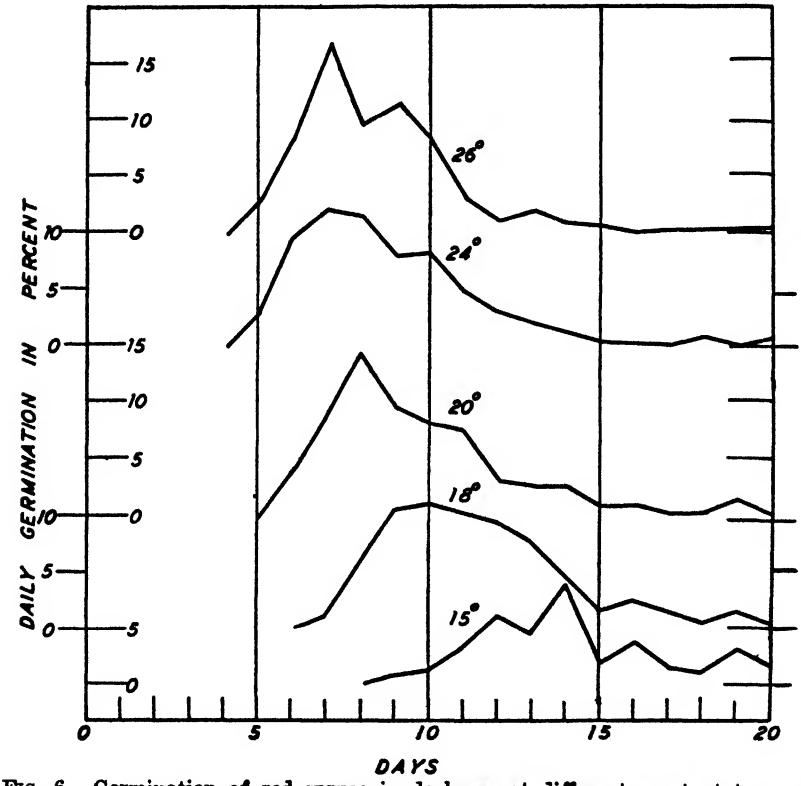


FIG. 6. Germination of red spruce in darkness at different constant temperatures (° C.), second series. No germination occurred at 10°. Basis 800 seeds each curve.

considerably later. The peak is usually reached by the 12th day, and germination is nearly over by the 20th day in germinators. A few seeds continue to germinate for 30–50 days (fig. 5). The beginning of germination is closely correlated with the rate of water absorption or imbibition. Seed coats of red spruce seeds imbibe water readily, as shown by the following experiment.

Two samples of 100 seeds each of the 1928 crop, containing 7 per cent. moisture based on dry weight, were placed in individual JACOBSEN germinators (2) with a water level 10 cm. below the plate. The seeds were weighed

on an analytical balance before being placed in the germinators, and at intervals until germination started. In weighing, the seeds were brushed carefully on to a dry filter paper which had been balanced on the pan, and the weighing made as rapidly as possible, to avoid loss by evaporation. A fresh paper was used for each sample. After the final weighing, the seeds

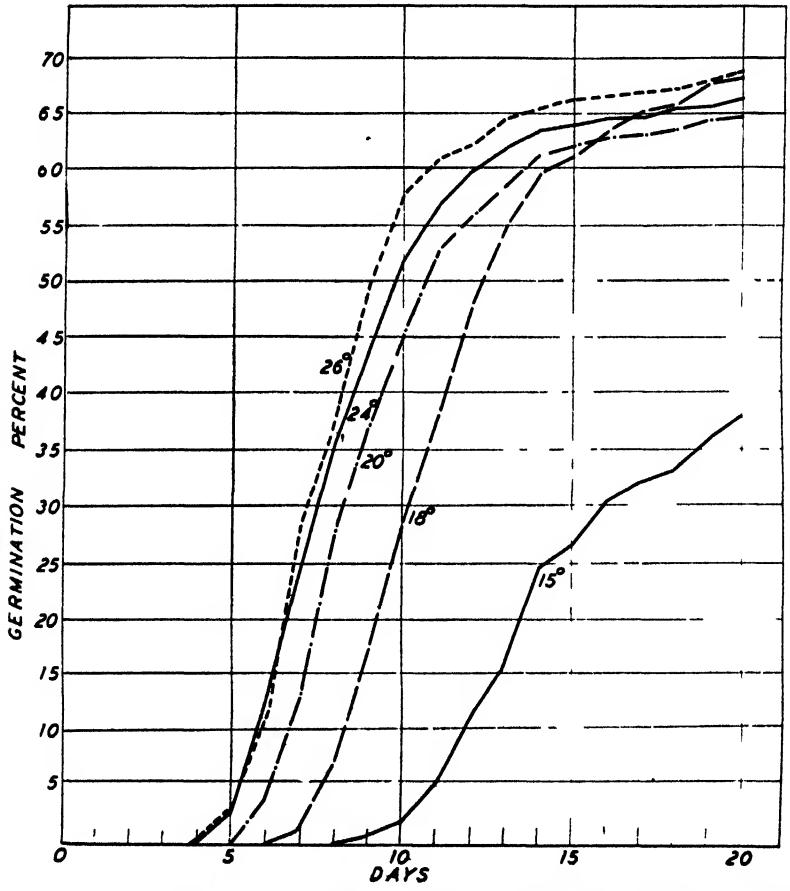


FIG. 7. Daily germination of red spruce seed at different constant temperatures (° C.), second series. Basis 800 seeds each curve.

were oven-dried at 105° C. for 48 hours and the dry weight determined. The moisture percentages are illustrated in figure 9. The rapid initial absorption demonstrated that the seed coats were readily permeable.

RATE OF WATER ABSORPTION AS RELATED TO VOLUME.—Upon imbibition, the volume of the seed plus that of the imbibed water increased. This is not true of all seeds. The behavior of red spruce seed was demonstrated by the following experiment:

A small bulb was blown in one end of a glass tube, sealed off and filled with air-dry seeds. The tube and bulb were filled with boiled distilled water, care being taken to eliminate air bubbles. The tube was then attached to a graduated manometer tube filled with mercury, similar to the apparatus described by DU SABLON (45). The height of the mercury column was read at intervals for two weeks, the apparatus standing on the laboratory desk in

FIG. 8. Apparatus in place for studying fall germination.

diffuse light at about 22° C. A continuous rise occurred, increasing to a maximum at seven days, and then remaining constant. The trend was as follows:

INCREASE IN VOLUME AFTER:					
4 hrs.	6 hrs.	28 hrs.	2 days	7 days	12 days
0.04 ml.	0.05 ml.	1.90 ml.	1.92 ml.	2.70 ml.	2.70 ml.

The rate of increase in both weight and volume due to imbibition of water will be seen to be closely correlated with the time elapsing before germina-

tion commenced. OSTERHOUT (38) found the rate of water absorption much greater at high temperatures than at low ones, and it seems probable that the hastening of germination observed at higher temperatures may be explained by the more rapid intake of water and the speeding of chemical reactions according to the van't Hoff law.

4. GERMINATION IN LIGHT AND DARKNESS.—The first experiments on the effect of light upon the germination of red spruce seeds were made in March, 1930, in the greenhouse of the Marsh Botanical Garden. Eight samples of 100 seeds each of the 1928 crop were placed in a dark chamber and eight in the light chamber. The seeds were germinated in petri dishes as in the

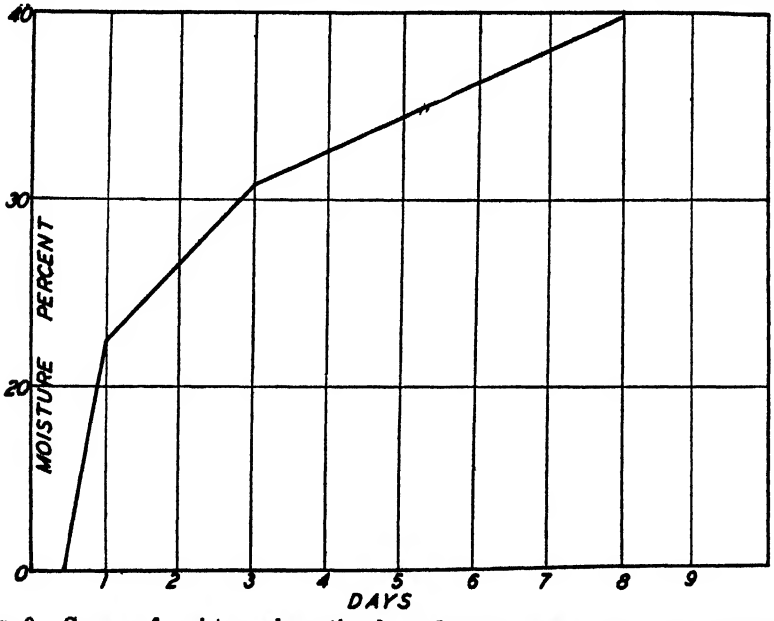


FIG. 9. Course of moisture absorption by red spruce seed prior to germination on JACOBSEN germinator.

experiments with temperature, and the apparatus for securing darkness was the same chamber as was used by DILLER (16) and HUTCHINGS (22) in their studies on the effect of light and darkness on germination. Sprouted seeds were counted and removed at intervals of five days. Final cutting tests were made on all seeds which did not germinate within 30 days. Temperatures in the chambers read with current thermometers at the time of counting the sprouted seeds varied from 21° to 24° C., which fell within the effective optimum range for normal germination (*q.v.* above).

The results are shown in table VI and in figure 10. After 10 days about 10 per cent. higher germination was recorded in darkness than in light;

hypocotyls were longer in the dark, possibly because of the absence of the inhibiting effect of light. After 15 days the difference between the light and dark samples had become less, and at 20 days the number germinated in light exceeded that in darkness. At 30 days the average germinations of the two series varied by but 1.5 per cent., and real germinations[2] showed a mean

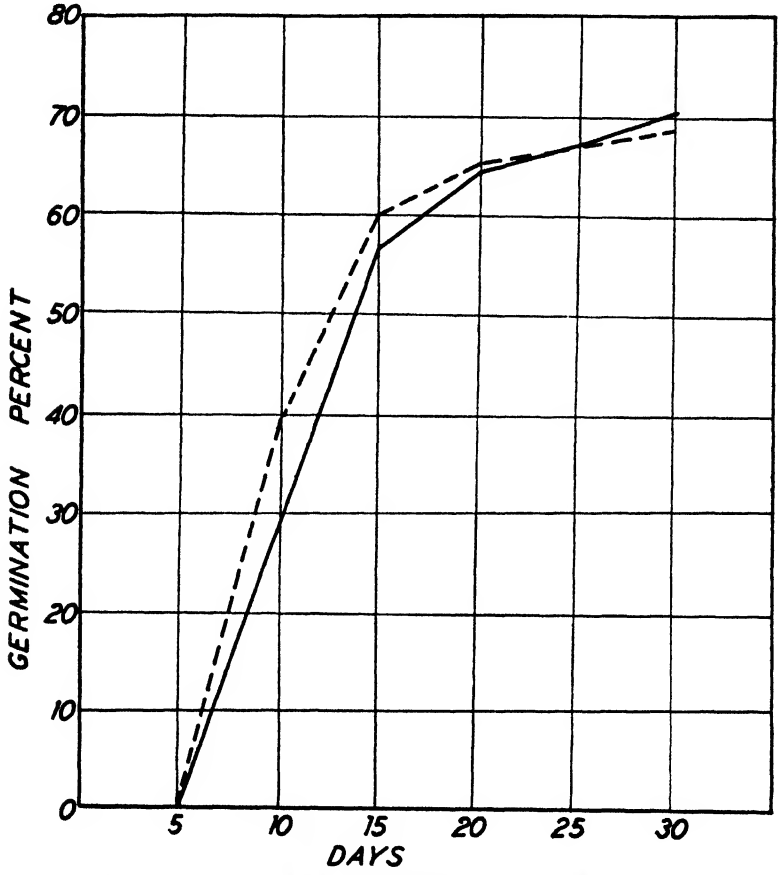


FIG. 10. Germination of red spruce in light and darkness. Dotted line indicates darkness. Basis 800 seeds each curve.

difference of but 0.8 per cent. A computation of average and standard deviations from the mean indicated that the two series exhibited essential uniformity, and no significant difference in behavior. Both standard deviations and probable error were greater in magnitude than the difference in germina-

[2] "Real germination" is based on the percentage of sound seeds germinating, and thus differs from the apparent germination of the entire sample, when many empty seeds are present.

TABLE VI

AVERAGE GERMINATION OF RED SPRUCE SEED IN LIGHT AND DARKNESS

YEAR COLLECTED	DATES TESTED	GERMINATION TEMPERATURE	APPARENT GERMINATION IN 30 DAYS		REAL GERMINATION (PER CENT. OF FILLED SEED GERMINATED IN 30 DAYS)		BASIS (NO. OF SEED)
			LIGHT	DARKNESS	LIGHT	DARKNESS	
		°C.	%	%	%	%	
1928	Mar. 1930	24	70.0	68.5	75.2	76.0	1,600
1929	Nov. 1930	"	80.5	69.2	82.0	70.0	800
"	Dec. 1930	"	77.2	69.0	79.4	70.5	800
"	Jan. 1931	"	71.2	62.2	73.0	63.2	800
"	Feb. 1931	"	80.5	60.7	81.0	62.2	800
"	Mar. 1931	"	69.0	51.5	74.3	56.1	800
"	(Av. of 20 monthly tests		70.34	54.17			16,000

tive energy between the light and dark series. The experimental error was thus greater than the observed difference between the two conditions.

Beginning in November, 1930, a series of tests was started, primarily to study periodicity of germination. Unfortunately, change of residence necessitated the abandonment of these tests in June, 1932, after 20 months. They give, however, abundant data on germination in light and in darkness. Four samples were set up in light and four in darkness on the JACOBSEN germinator at 24° C. on the first of each month. Darkness was secured by coating the inside of the glass bell-jars with black enamel and India ink, while the outer surfaces were painted with white enamel to prevent absorption of heat. Thermometers were inserted in both dark and light samples and read daily. The temperatures were practically identical under both conditions, and, since temperature was controlled thermostatically, no fluctuations occurred. The results are given in table VI. They show from 10 to 30 per cent. greater germination in 30 days in light than in darkness, and suggest that light may be definitely beneficial in hastening germination, although by no means essential.

After-ripening.—Since red spruce sheds much of its seed in the autumn, which then remains on the wet ground under the snow, or in contact with melting snow until late spring before germinating, it might be supposed that cool moist storage of the seed would be the natural condition, to which the seed had become accustomed for centuries, and that stratification under such conditions would have the effect of hastening germination. To test this the following experiments were made:

TABLE VII

AVERAGE GERMINATION OF 1½ YR.-OLD RED SPRUCE SEED WITH AND WITHOUT AFTER-RIPENING

DATE COLLECTED	DATE TESTED	AFTER RIPENING TEMPERATURE	PERIOD OF AFTER RIPENING	GERMINATIVE ENERGY IN 30 DAYS		GERMINATION TEMPERATURE		BASIS NO. OF SEED
				GERMINATOR	SOIL	GERMINATOR	SOIL	
		°C.	*months*	%	%	°C.	°C.	
October 1928	February–March 1930	10	2	69	71	15–20	10–35	300
		10	2.5	83.5	77	15–20	10–35	700
		Controls		79.5	47.5	.....	.....	700
October 1929	February–March 1931	7	1	73.1	.....	26	.....	800
		Control	...	67.1	.....	26	.....	800
		7	2	70.6	.....	26	.....	800
		Control	...	50.1	.....	26	.....	800

Seed of the 1928 lot was stratified in moist peat at 8°–10° C. for approximately two months. Part of the seed was then removed and germinated along with similar samples of untreated seed, in individual JACOBSEN germinators. The germination temperature varied from 15° at night to 20°–25° C. by day. Part of the stratified seed was allowed to dry for two weeks. The remainder was dug up and samples of the freshly dug seed, seed which had been dried two weeks after stratification, and unstratified controls were germinated in soil flats in the greenhouse. The flats were prepared as described by TOUMEY and STEVENS (**58**). The sprouted seeds were counted and removed at 5-day intervals.

The results are given in table VII and shown graphically in figures 11, 12. In every case moist storage at low temperatures resulted in an increase in germinative energy and capacity as well. The differences, however, between the courses of germination of stratified and unstratified seeds were in no case as great as found for some other conifers by other investigators (**44, 39, 59, 60, 5, 6** and others), or by the writer (**3**) for eastern hemlock (*Tsuga canadensis* (L.) Carrière.)

In December, 1930, another series of experiments on after-ripening was started. Seed of the 1928 crop was used. One hundred seeds were placed on a layer of moist granulated peat 1 cm. thick in each petri dish, and covered with a thin layer of powdered peat. The dishes were then stored in a cold cellar at 6°–8° C. At the end of one month and of two months eight samples each were removed and placed at 24° C. to germinate along with eight similar samples of dry seed, freshly placed in peat as controls. Every five days the dishes were examined for sprouted seed and the peat worked over with tweezers to insure proper aeration. Water was added if necessary. The summarized results are shown in table VII and figures 11, 12. No significant difference in germination was observed between the two sets of samples. What little hastening of germination occurred in the stratified samples can be explained by the fact that these seeds were already fully imbibed as compared with the dry seeds.

SUMMARY OF RESULTS OF AFTER-RIPENING EXPERIMENTS

Field observations by a number of workers, as well as laboratory tests, show that seeds of several species of spruce cannot be classed as requiring prolonged after-ripening before becoming capable of normal germination. The present experiments confirm this conclusion; germination was hastened, but to a slight degree only.

5. GERMINATION UNDER AERATING WATER.—It is generally believed that excessively moist seed-beds are unfavorable for germination, the poor results obtained under such conditions being usually attributed to lack of

aeration. When seeds swell in water their respiration rate increases, and if sufficient oxygen is not at hand death ensues. TOUMEY and DURLAND (54) found that seed of upland species soaked over five days in water at greenhouse temperature gave very low germination, if it germinated at all.

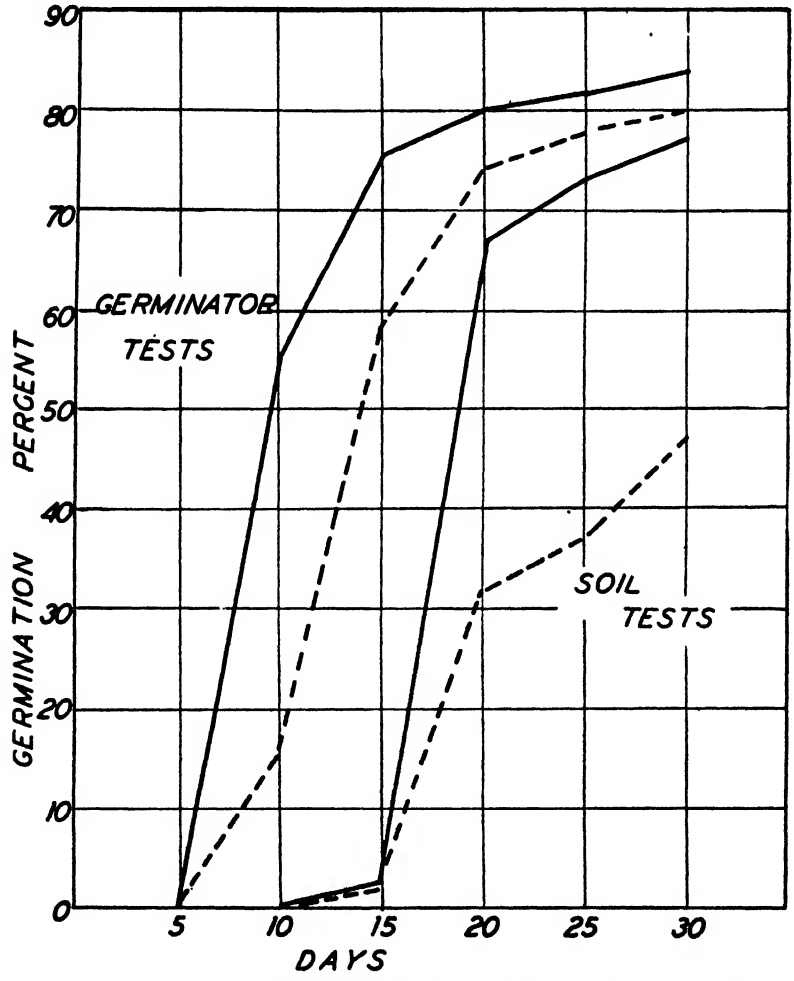


FIG. 11. Germination of after-ripened seed of red spruce. Solid lines represent seed stratified 75 days at 10° C. Dashed lines, controls. Basis 1700 seeds.

With white spruce the germination fell off constantly with longer soaking, until no germination was secured after 30 days. Red spruce was not tested. In the present experiments the seeds were kept totally immersed and germination in aerated and unaerated water compared.

Two hundred seeds of the 1928 crop were attached to both sides (100 on a side) of a glass strip 2 cm. wide, by coating the strip with shellac and

pressing the seeds into it. The glass strip was inserted into a cork fitting the neck of a 2-liter bottle and suspended vertically in the bottle. Two such bottles were set up and filled completely with water which had been boiled to expel all dissolved air and then cooled. One bottle was arranged to be constantly aerated by blowing a stream of air through it from a compressed air tap, leading the air to the bottom of the bottle through a glass

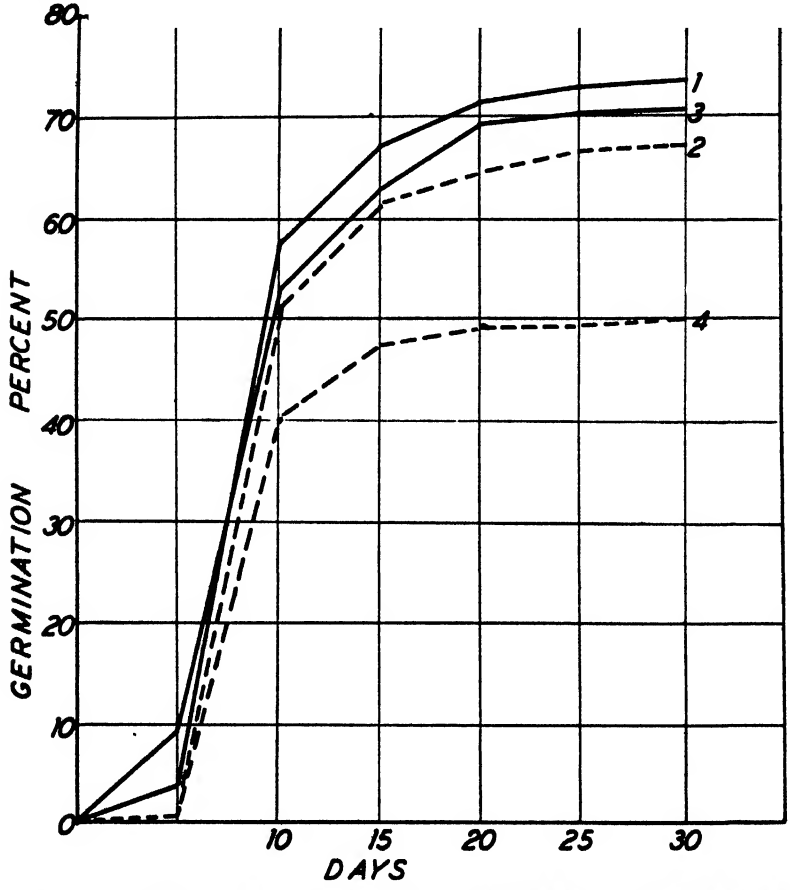


FIG. 12. Germination of after-ripened seed of red spruce: (1) Stratified one month at 7° C. (2) Control. (3) Stratified two months at 7° C. (4) Control (basis 800 seeds each curve.)

tube. The other bottle was not aerated. The bottles were kept at room temperature, averaging about 23° C.

After nine days some seeds in the aerated bottle began to germinate, and 13 days after the start of the experiment, aeration of this bottle was discontinued and the control bottle attached to the air tap. Up to this time

no germination had occurred in the control bottle. Six days after aeration of the control bottle had begun, some seeds were noticeably swollen, and after 11 days about 10 per cent. had germinated. This showed that even seeds soaked 13 days in the absence of oxygen retained their vitality.

Final counts from both bottles were as follows (basis of 200 seeds):

(1) Dry seed placed in water and aerated 13 days ..... 57 per cent.
(2) Dry seed placed in water without aeration ..... 0 per cent.
(2) after subsequent aeration for 13 days ..... 28 per cent.

These experiments suggest that red spruce seed is capable of enduring considerable soaking and storage under anaerobic conditions without losing its viability. Presumably submergence at lower temperatures than those used in these experiments, and such as would occur in nature, would have an even less deleterious effect. Submergence at higher temperatures would shorten the period the seeds would remain viable, as shown in TOUMEY and DURLAND'S experiments (**54**).

TABLE VIII

GERMINATION OF RED SPRUCE SEED IN SOLUTIONS OF DIFFERENT pH
GERMINATIVE ENERGY AT 21 DAYS. EACH FIGURE IS THE AVERAGE OF 200 SEEDS

pH	SERIES AND DATE							
	I FEB. '28	II*—III MAY '28	IV OCT. '28	V DEC. '28	VI MAR. '29	VII APR. '29	VIII†—IX† SEPT. '29	
	%	%	%	%	%	%	%	%
1.05					0	0		
2.05					**70**	44		
2.5					67	64		
3.0		**60**		51	54	**56‡** **59‡**		
3.5		49		44	58	56		
4.0		47		**56**	51	73.5		**71**
4.5		49		56	molded:	unreliable*	75	46
5.0		40		34	"	52	77	54.5
5.5	**88**	52	**26**	26	43	48		
6.0	83	41	**26**	44	57	**47‡** **19‡**		
6.5	76	54	17	34	31	30		
7.0	19	33	6	41	33	25	**77.5**	60.5
7.5								
8.0	17	35			1.5	41	70.5	65
8.5							51.5	3.5
Controls (distilled water)	88	51	35	24	66	52	77.5	73.5

* Results unreliable owing to excessive mold; results not given.

† Figures give germination at 19 days for series VIII and 17 days for series IX respectively.

‡ Two overlapping solutions of same pH.

Figures in bold face type indicate best germinations for each series of tests.

GERMINATION IN DIFFERENT HYDROGEN ION CONCENTRATIONS.—Several students of spruce reproduction have attached importance to the acidity of the seed-bed as a factor controlling the establishment of spruce. Field observations showed that seedlings grew in acid habitats. Table IX gives

TABLE IX

REACTION OF SOILS IN WHICH SPRUCE SEEDLINGS WERE GROWING

CHARACTER OF HUMUS	pH VALUE	MONTH
Northern hardwood leaf mold	6.5	May
Birch poplar leaf mold	6.5	May
Mineral soil under northern hardwoods	6.8	May
Red spruce humus (duff)	6.5	November
Raw humus under young spruce stand	4.8	April
Dry undecomposed spruce needles	4.3	October

the average pH values of soil clinging to roots of spruce seedlings a few months to a year old on various sites. They were averaged from a large number of determinations made near Cupsuptic Lake, Maine, in 1924 and 1925, using a LaMotte indicator field set.

An optimum degree of acidity for the germination of Norway spruce (*Picea excelsa* Link.) has been noted by AALTONEN (1) and SCHMIDT (48). The latter germinated seed in buffered solutions, and pointed out the danger of the results being influenced by the character of the solution unless overlapping tests were made with different salts to secure the desired pH range. He found the use of a single acid, such as sulphuric acid, unsuitable owing to the instability of an unbuffered solution.

Laboratory experiments were begun in February, 1928, and extended over 20 months. All-glass individual JACOBSEN germinators were used. Special care was taken in the cleaning of the glassware. It was soaked in acid bichromate cleaning fluid, rinsed, boiled in soap suds, rinsed in distilled water, and dried. The wicks were of unused pure cotton yarn. They were boiled in distilled water, and then each soaked in its corresponding solution before being put in place. High grade filter papers were placed on the wicks. Eight hundred-ml. pyrex beakers were used as reservoirs, filled to within 7.5 cm. in the first series, and to 10 cm. of the top in later experiments. After setting up the apparatus, the beakers were placed in the water bath of the large JACOBSEN germinator (fig. 2) and maintained at a constant temperature of 24° C. Two samples of 100 seeds each were set up for each different pH, and for each different solution. The same lot of seed of the 1927 crop was used in all tests. In series VI and VII the seed was counted with a special suction counter described by BROWN, TOOLE, and GOSS (10) and lent by courtesy of the Seed Laboratory, U. S. Depart-

ment of Agriculture. Wherever possible the ranges of different solutions were made to overlap 1 0 pH. Solutions were prepared varying by 0.5 pH from pH 1.0 to 8.5. The substances used for buffers included succinic acid, borax, sodium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, sodium chloride, citric acid, and glycocol. Before being used all solutions were diluted the same amount with distilled water, and their pH checked electrometrically by the calomel electrode method. At the end of the test the reactions of the filter papers were checked. A small quantity of liquid was sucked out of the filter paper with a dropper and its pH determined colorimetrically. In no case was any change detected, except in the last series, where the method of germination differed and the solutions were exposed to the air, and so could absorb CO_2. The experiments were repeated eight times. Results are presented in table VIII.

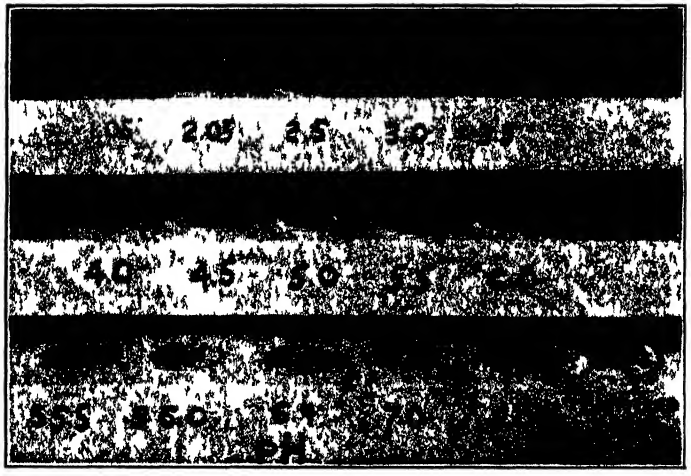


FIG. 13. Red spruce seedlings germinated in solutions of different pH.

In the first five series measurements were made of the lengths of tops and roots of seedlings in each sample. Twenty-five or more seedlings were drawn at random from each sample and measured on a millimeter scale. They were then weighed and the weight of 50 seedlings in grams computed for each sample.

In all the series, striking differences were observed in the germination and growth over different hydrogen-ion concentrations. These were apparent after the first week. The first seeds to germinate were invariably in the more acid samples in the range pH 3.0–5.5. Neutral and alkaline tests showed retardation in germination at the start, and after three weeks the seedlings had made much poorer growth than those on acid samples, or had died. Mold attack was most severe in the case of neutral and alka-

line samples. Solutions of salts in most cases seemed to have an unfavorable effect upon germination as compared with distilled water, but the effect of acidity could be noted in samples of the same solution with different pH values. In some cases better germination was secured in the buffered solutions than in the distilled water controls. The detailed tables showing courses of germination under the different conditions and data on weights of seedlings are omitted on account of lack of space. The appearance of the seedlings as shown in figure 13 gives a much better idea of the effect of the various degrees of acidity than would the numerical data.

Summary of results of germination in different hydrogen-ion concentrations

Germination was found to take place more rapidly and completely in the more acid solutions than in neutral and alkaline ones. In most cases alkaline solutions proved distinctly injurious. The range of tolerance on the acid side was very wide; good germination was obtained from pH 2.0 to 6.6. The superiority of highly acid substrata may have been due in some degree to the greater freedom from mold. A pronounced optimum pH could not be found. In general red spruce seeds germinated best between pH 3.5 and 5.5. Figure 14 presents some of the results graphically.

Discussion of results

The experiments with different incubation temperatures showed that germination occurred at a wide range of temperature. Thirty-eight degrees C. was found definitely too high, which agrees with the findings of Haasis (19). If the seeds could have been kept perfectly sterile it is possible that some might have germinated. Thirty-two degrees was above the optimum, which probably lies between 24° and 26° C. The differences in germination between any temperature from 18° to 28° C. were chiefly due to hastening germination in the early stages of incubation by faster water absorption and chemical changes in the seed. The end results after 20 days were nearly identical. Figs. 5–7 show clearly the influence of higher temperatures in hastening germination, in crowding the bulk of the germination into a shorter period, and in causing it to begin earlier. Apparently germination can occur a few degrees above freezing if the seed has been stored long enough previously at low temperatures. The lower the temperature, the more important becomes the time factor, and the period of germination is enormously extended at low temperatures, as observed by Haasis (19). No germination occurred at 10° C. after one month's trial.

The effectiveness of long incubation at low temperatures in lowering the temperature requirements has an important bearing on the after-ripening experiments. In these tests after-ripening was found to have little effect

on the rate of germination at relatively high temperatures, the optimum range for untreated seed. The capacity of the seed to germinate at low temperatures also helps to explain its failure to remain viable in the duff, assuming, of course, that moisture is adequate.

In spite of the more or less negative results of the first experiments on light and darkness, the later experiments seem to indicate that germination

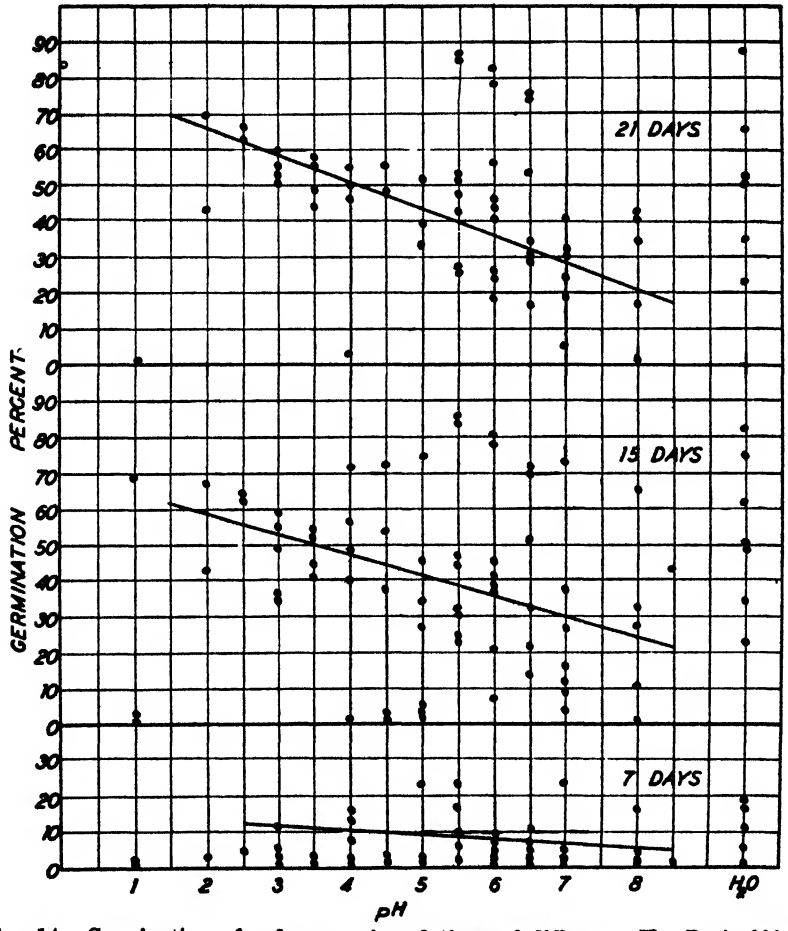


FIG. 14. Germination of red spruce in solutions of different pH. Basis 200 seeds each dot.

is hastened somewhat by light. In the first series the average germination after 30 days in light and darkness varied but little. In the 20 monthly tests carried out subsequently, dark germination exceeded light in but one case, and the excess of light over dark was generally 10 per cent., and once or twice about 30 per cent. Lack of light seems to retard the rate of germi-

nation, not to prevent it. Light probably acts as a stimulant to set in motion the chemical changes preliminary to germination.

The effect of the combined action of light and temperature, especially low temperature, was not investigated. With a species which germinates as readily as red spruce, it seems doubtful whether the influence of such factors would be profound. In most cases where the combined action of light and low temperature (or darkness and low temperature) has been found effective in hastening germination, the seed germinated with great tardiness under ordinary germinator conditions (KINZEL **25–29**).

The rôle of oxygen in the germination of red spruce seeds may be important when the seeds are imbedded under hardwood leaves or mulch. While starchy seeds, such as peas and beans, can be germinated anaerobically because of the large amount of oxygen present in their storage foods, fatty seeds like spruce require oxygen from outside in order to oxidize their fatty reserves for use in germination. However, the oxygen requirements of spruce seeds appear quite minimal. More detailed experiments would be necessary in order to determine quantitatively the limits under which germination would occur.

The promptness of germination in red spruce seeds seems to be due to two chief causes: (1) their physiological readiness and (2) their comparative ease of moisture absorption. Seeds which are not markedly dormant must germinate when external conditions are favorable; if not, the chemicosynthetic processes are so weakened that they often cannot repeat the awakening. The inability of the seed to lie dormant over occasional periods of warm weather after seed-fall results at times in the devastating autumn germination of red spruce alluded to by many writers.

Water is the most important single factor in germination within the favorable range of temperature. Water, besides causing hydrolysis of the reserves in the seed, is responsible for the more or less mechanical swelling and bursting of the seed coat.

The short period that spruce seed was found to remain dormant in the duff can be interpreted in this light: conditions even approaching the minimum for germination mobilize the ferments; if the stimuli are then removed, the seed is weakened by the expenditure of energy, and will respond less vigorously on the next occasion. It is even more probable that it will actually germinate and be unable to reach the surface, or survive adverse climatic conditions. In examining seeds removed from the duff, black fungous threads were often observed enveloping them. HARTLEY, MERRILL, and RHOADS (**21**) state that considerable quantities of seed are destroyed by ordinary saprophytic molds, and suggest that failure to germinate in humus and leaf mold might be due to the attack of molds and even to damping off fungi like *Corticium vagum* B. & C. or *Pythium debaryanum*

Hesse. That seeds are killed by fungi before the coats are split has recently been demonstrated by RATHBUN-GRAVATT (43).

The experiments on germination in different hydrogen ion concentrations, in spite of inconsistent results in many cases, confirm fairly well the conclusions of other investigators that an acid reaction is not unfavorable to spruce seed; in fact, indications are that acid conditions more or less favor germination. If the illustrations of the seedlings at the end of 21 days (fig. 13) are compared with the corresponding figures for germination, it will be observed that the effect of the acid is more evident from the appearance of the seedlings than from the germination statistics. This agrees with the findings of SALTER and MCILVAINE (46) and SCHMIDT (48). It would appear that the seeds can germinate well from near neutral to pH 2.0. No data were obtained on mortality following germination, but seedlings growing in alkaline solutions died soon after germination, or exhibited blackening of the roots. From the curves for the course of germination it will be seen that the lower pH values not only permitted a greater final germinative energy in the period, but apparently stimulated earlier germination. This may account for the greater percentage of seeds germinating before being attacked by mold. While the controls over distilled water usually gave larger and more healthy seedlings, higher germination percentages were often recorded for the strongly acid samples. A comparison of the development of red spruce seeds in different reactions under laboratory conditions with those under the different acidities found in the field confirms their tolerance of a wide range of pH values.

These studies of the germination requirements show that no very narrow limits control germination; all requirements are met under average conditions during the growing season. Moisture, then, must be the limiting factor for germination much of the time. Probably most viable spruce seeds germinate promptly in the spring, but few seedlings survive. Lack of reproduction is more likely due to unsuitable conditions for survival and establishment than failure to germinate, provided of course that the seed supply is adequate.

Summary

1. Red spruce seeds were capable of germinating promptly following maturity under a wide range of conditions.

2. In the field, germination and survival were best on mineral soil.

3. Germination and survival were favored by surface soil moisture.

4. Under the conditions of the experiments the seed did not retain its vitality long when stored in the duff. Most of the seed germinated in the duff during the first season. A small percentage remained viable for one year, and occasionally for two.

5. The average germinative energy of freshly harvested seed was found to be 66–87 per cent. in 30 days, with an average of 77 per cent. During air-tight dry storage it decreased about 10 per cent. per year for the first three years.

6. Germination of air-dry seed took place within a temperature range of 15°–32° C. Germination was incomplete below 20° and above 28° C. The optimum range appeared to be 24°–26° C.

7. After prolonged stratification at low temperatures, the minimum temperature for germination was lowered.

8. After-ripening at 7°–10° C. in a moist substratum hastened germination only slightly.

9. Light was found non-essential, but somewhat stimulating to germination.

10. Moisture was most important, especially at the beginning of germination. Moisture absorption was rapid, and began immediately.

11. Germination took place under water when aeration was provided.

12. Red spruce seeds tolerated a wide range of acidity, and germinated well between pH 2.0 and 7.0. Germination increased up to pH 2.0 and was poorest toward the neutral point and alkaline side. Best seedlings resulted between pH 5 and 6.

13. The speed of germination was greater in the more acid samples.

14. The most vigorous seedling growth was obtained in the distilled water controls, but there was no evidence that the buffered solutions used retarded germination.

15. Molding of the seed was less severe at higher acidities.

16. These experiments indicate that natural reproduction of red spruce seeds is dependent not so much on the requirements for germination as on the factors which determine survival of the seedlings after germination takes place.

This investigation was made possible by cooperation between the School of Forestry, Yale University, and the Brown Company, Portland, Maine. Field studies were begun at Cupsuptic Lake, Maine, in 1924 and were continued at Berlin, N. H., until June, 1932. Laboratory work has been carried on at the Forest Investigations Laboratory of the Brown Company Forestry Division. The writer is especially indebted to the late Dr. JAMES W. TOUMEY, Professor of Silviculture in the Yale School of Forestry, under whose direction the investigations were carried out, for many helpful suggestions in planning and carrying out the studies, and in the analysis of the data and preparation of the original manuscript. He desires also to express his sincere appreciation to Mr. W. R. BROWN, for permitting the publication of the parts of the investigation carried on by the writer while employed by the Brown Company. Thanks are also extended to Professor

Dr. WERNER SCHMIDT, Leiter, Waldsamenprüfungsanstalt, Eberswalde, Germany, for much kindly counsel, and for so generously placing the resources of his Station at the writer's disposal; to Dr. GEORGE E. NICHOLS for laboratory facilities at the Osborn Botanical Laboratory, Yale University; Dr. LORANDE L. WOODRUFF of the Osborn Biological Laboratory for use of constant temperature ovens; Dr. CARL G. DEUBER for many constructive suggestions; Dr. JOHN S. BOYCE for the identification of molds; Dr. WILLIAM CROCKER, Director of the Boyce Thompson Institute for Plant Research, for examining seed from duff samples, and for many helpful suggestions; and to Mr. P. S. GLASSON of the Brown Company Research Department for preparing solutions of different pH value.

THE CAROLINE A. FOX RESEARCH AND DEMONSTRATION FOREST
STATE OF NEW HAMPSHIRE, FORESTRY DEPARTMENT
HILLSBORO, N. H.

LITERATURE CITED

1. AALTONEN, V. T. Über den Aziditätsgrad des Waldbodens. Medd. fr. forstvetenskapliga Försöksanstalten **9**: 26–29. 1925.
2. BALDWIN, H. I. An individual JACOBSEN germinator. Science n. s. **72**: 345–346. 1930.
3. ———. The effect of after-ripening treatment on the germination of eastern hemlock seed. Jour. For. **28**: 853–857. 1930.
4. BARR, P. M. The effect of soil moisture on the establishment of spruce reproduction in British Columbia. Bull. 26, School of Forestry, Yale University. 1930.
5. BARTON, L. V. Hastening the germination of southern pine seeds. Jour. For. **26**: 774–785. 1928.
6. ———. Hastening the germination of some coniferous seeds. Amer. Jour. Bot. **27**: 88–115. 1930.
7. BOERKER, R. H. Ecological investigations upon the germination and early growth of forest trees. University Studies, University of Nebraska. **16**: 1–89. 1916.
8. BOYCE, C. W. Recent trends in the paper industry. Pulpwood **3**: 9. 1930.
9. BRANDTS, P. De kieming der zaden. Natuurw. Tijdschr. **4**: 233–234. 1922.
10. BROWN, E., TOOLE, E. H., and GROSS, W. L. A seed counter. Circl. 53, U. S. Dept. Agr. 1928.
11. BUELL, J. H. (Cited by TOUMEY and KORSTIAN) 1931.
12. CARY, N. L. Sitka spruce, its uses, growth, and management. Bull. 1060. U. S. Dept. Agr. 1922.
13. CHITTENDEN, A. K. Forest conditions in northern New Hampshire. Bull. 55. Bur. Forestry, U. S. Dept. Agr. 1905.

14. CROCKER, W. The mechanics of dormancy in seeds. Amer. Jour. Bot. **3**: 99–120. 1916.
15. ————. Points of agronomic interest in the physiology of germination. Jour. Amer. Soc. Agron. **17**: 696–705. 1925.
16. DILLER, J. D. The effect of light on the germination of certain seeds. MS. Thesis, School of Forestry, Yale University. 1930.
17. FAHRENBACH, J. H. A report on the fruit, seed, and germination of 25 species of forest trees. MS. Yale Forest School. 1910.
18. GRAVES, H. S. Practical forestry in the Adirondacks. Bull. 26. Div. of Forestry, U. S. Dept. Agr. 1899.
19. HAASIS, F. W. Germinative energy of lots of coniferous tree seed as related to incubation temperature and to duration of incubation. Plant Physiol. **3**: 367–412. 1928.
20. HAIG, I. T. Premature germination of forest tree seed during natural storage in duff. Ecology **13**: 311–312. 1932.
21. HARTLEY, C., MERRILL, T. C., and RHOADS, A. S. Seedling diseases of conifers. Jour. Agr. Res. **15**: 521–558. 1918.
22. HUTCHINGS, S. S. Light in relation to the seed germination of *Mimulus ringens* L. Amer. Jour. Bot. **19**: 632–643. 1932.
23. JACOBSEN, I. Oversigt over Resultater af Undersøgelser af Traefrø. Dansk Skovfor. Tidsskr. 724–742. 1926.
24. JOSEPH, H. C. Germination and vitality of birch seeds. Bot. Gaz. **87**: 127–151. 1929.
25. KINZEL, W. Lichtkeimung. Ber. deutsch. bot. Ges. **26**: 631–645. 1908.
26. ————. Frost und Licht als beeinflussende Kräfte bei der Samenkeimung. Stuttgart. 1913.
27. ————. Frost und Licht. Abschluss der Erläuterungen und Ergänzungen zum ersten Buch. Nachtrag II. Stuttgart. 1920.
28. ————. Grenzen der förderlichen einwirkung von Frost und Licht bei der Samenkeimung. Angew. Bot. **12**: 16–22. 1930.
29. ————. Höhenkeimer. Angew. Bot. **14**: 182–193. 1932.
30. KORSTIAN, C. F. Factors controlling germination and early survival in oaks. Bull. 19. School of Forestry, Yale University. 1927.
31. LI, T. T. Soil temperature as influenced by forest cover. Bull. 18. School of Forestry, Yale University. 1926.
32. MOORE, B. Reproduction in the coniferous forests of northern New England. Bot. Gaz. **64**: 149–158. 1917.
33. ————. Some factors influencing the reproduction of red spruce, balsam fir, and white pine. Jour. For. **15**: 827–853. 1917.
34. MULLOY, G. A. Silvicultural problems of our balsam fir-spruce type forests. Canadian Woodlands Rev. **1**: 9–10. 1930.

35. MURPHY, L. S. Seeding habits of spruce as a factor in the competition of spruce with its associates. Plant World **20**: 87–90. 1917.
36. ————. The red spruce: its growth and management. Bull. 544. U. S. Dept. Agr. 1917.
37. ————, and ZON, R. Seeding habits as a factor in competition between spruce and balsam fir. Jour. Ecol. **5**: 127. 1917.
38. OSTERHOUT, W. J. V. Some aspects of the temperature coefficients of life processes. Jour. Biol. Chem. **32**: 23–27. 1927.
39. PACK, D. A. The chemistry of after-ripening, germination, and seedling development of juniper seeds. Bot. Gaz. **72**: 139–150. 1921.
40. PETTIS, C. R., and LUMMIS, G. M. How to grow and plant conifers in the northeastern states. Bull. 76. U. S. Forest Service. 1909.
41. PINCHOT, G. The Adirondack spruce. The Critic Co., New York. 1898.
42. RAFN, J. The testing of forest seeds during 25 years. Copenhagen. 1912.
43. RATHBUN-GRAVATT, A. Germination loss of coniferous seeds due to parasites. Jour. Agr. Res. **42**: 71–92. 1931.
44. ROSE, D. H. A study of delayed germination in economic seeds. Bot. Gaz. **59**: 425–444. 1915.
45. DU SABLON, L. Traité de physiologie vegetale et agricole. Paris. 1911.
46. SALTER, R. M., and MCILVAINE, T. C. Effect of reaction of solution on germination of seeds and on growth of seedlings. Jour. Agr. Res. **19**: 73–96. 1920.
47. SARGENT, C. S. Silva of North America **12**: 34. 1898.
48. SCHMIDT, W. Reaktionsempfindlichkeit von Keimlingen. Forstarchiv **3**: 81–82. 1927.
49. ————. Unsere Kenntnis vom Forstsaatgut. Berlin. 1930.
50. STETSON, I. G. A study of the seeds and seedlings of 42 arborescent species. MS. Yale Forest School. 1909.
51. SUDWORTH, G. B. The forest nursery. Bull. 29. Div. Forestry, U. S. Dept. Agr. 1900.
52. TILLOTSON, C. R. Growing and planting coniferous trees on the farm. Farmers Bull. 1453. U. S. Dept. Agr. 1915.
53. TOUMEY, J. W. Seeding and planting in the practice of forestry. 1st ed. New York. 1916.
54. ————, and DURLAND, W. D. The effect of soaking certain tree seeds in water at greenhouse temperatures on viability and the time required for germination. Jour. For. **21**: 269–275. 1923.
55. ————, and KIENHOLZ, R. Trenched plots under forest canopies. Bull. 30. School of Forestry, Yale University. 1931.

56. ————————, and KORSTIAN, C. F. Seeding and planting in the practice of forestry. 2d ed. New York. 1931.
57. ————————, and NEETHLING, E. J. Insolation a factor in the natural regeneration of certain conifers. Bull. 11. School of Forestry, Yale University. 1924.
58. ————————, and STEVENS, C. L. The testing of coniferous tree seeds at the School of Forestry, Yale University, 1906–1926. Bull. 21. School of Forestry, Yale University. 1928.
59. WAHLENBERG, W. G. Circumventing delayed germination in the nursery. Jour. For. **22**: 574–575. 1924.
60. ————————. Fall sowing and delayed germination of western white pine seed. Jour. Agr. Res. **28**: 1127–1131. 1924.
61. WESTVELD, M. Logging damage to advance spruce and fir reproduction. Jour. For. **24**: 579–582. 1926.
62. ————————. Spruce regeneration in eastern Canada and northeastern U. S. Forestry Chron. **6**: 22–33. 1930.
63. ANONYMOUS. Swift decay of Douglas fir seed on floor of virgin forest. For. Worker **7**: 9. 1931.

CARBOHYDRATE AND NITROGEN RELATIONS IN WHEAT PLANTS WITH REFERENCE TO TYPE OF GROWTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

ANNIE M. HURD-KARRER AND ALLAN D. DICKSON

(WITH FOUR FIGURES)

Introduction

The growth habit of the wheat plant is strikingly affected by relatively small changes in the environment. If the short days of winter are lengthened by a few hours of artificial illumination, the young plants of Turkey, a winter variety, grow erect instead of assuming the recumbent growth habit, which in winter sowings normally characterizes the early tillering stage (**28**). Also, if this variety is grown at temperatures above 20° C., culm elongation may be completely inhibited, resulting in the stunted indefinitely vegetative growth characteristic of spring-sown plants (**4**). Morphological characteristics of spring varieties, such as Hard Federation, which are less susceptible to modification by small temperature differences, are greatly affected by differences in day length. By increasing the natural length of the day through artificial illumination, the plants may be so rapidly forced into heading that at maturity they are extremely stunted, with reduced tillering, short spindling culms, and small leaves and heads. By decreasing the length of the light period, large vegetative plants are produced, with increased tillering but reduced yields of grain (**29**). Such variations in type of growth would seem to reflect correlative differences in the chemical organization of the plant.

Early investigators (**43, 30, 31**) attributed the transition from vegetative to reproductive growth to the accumulation of some unnamed organic material acting as a stimulus to flowering. The suggestions followed that the transition is brought about by the accumulation of carbohydrates (**32, 35**) or by an excess of carbohydrates over nitrogenous compounds (**14, 15, 34**).

The relative amounts of carbon and nitrogen compounds in the tissues have been reported to determine many aspects of plant behavior. Not only the general condition of fruitfulness, and of contrasting vegetative development, but also the rate and amount of growth, root development, relation of size of tops to that of roots, resting periods, fruit-bud differentiation, and the phenomena involved in "photoperiodism" have been reported to be correlated with the balance between carbon and nitrogen compounds in the tissues (**3, 23, 33, 46**). The analytical work on wheat includes that of

REID (41, 42), who showed that the type of seedling growth is determined by the relative amounts of available carbon and nitrogen compounds; and of HICKS (21, 22), who concluded that successive stages of development are initiated by critical values of the carbon/nitrogen ratio, flowering being brought about by a high C/N value.

The purpose of the present investigation was to extend these observations to a chemical characterization of the widely differing growth responses shown by the wheat plant when grown under various conditions of day length and temperature. By growing the winter variety, Turkey, and a spring variety, Hard Federation, under a number of controlled greenhouse environments, many different types of growth were obtained, ranging from the complete sterility of entirely vegetative plants, and the partial sterility resulting from extreme forcing conditions, to normal vigor and grain development. The data obtained are discussed from the standpoint of the direct effects of day length, temperature, light intensity and soil reaction, and also with reference to correlations of chemical composition with type of growth and fruitfulness.

Methods

1. Environmental conditions

Seeds of the varieties Hard Federation (C. I. 4733) and Turkey (C. I. 1558), representing respectively extreme types of spring and winter wheats, were sown on December 13, 1929, in each of two similarly situated greenhouses with automatically controlled temperatures. One house was kept at 10°–12° C., a temperature favorable for wheat, the other at 21°–23° C., which is unfavorably high. This high temperature was especially unfavorable for the development of Turkey, whose heading was largely inhibited above 20° C. Temperature control was satisfactorily maintained until the second week of April, when outdoor temperatures became too high. After this date an average difference of 5° C. in the temperature of the two houses was maintained by means of a sprinkler system on the roof of the low-temperature house.

The soil used was a clay-loam compost, 10 inches deep, thoroughly mixed to insure uniformity. Soil moisture was kept as uniform as possible in all the benches.

The plants of the first bench in each house were given a shortened day by means of a heavy black sateen cover, which was in place from 4 P. M. until 8 A. M. the next morning, giving the plants a light period of 8 hours in the middle of the day. The cover was adjusted over a framework to which it was secured at the corners by snap fasteners. It was removed by rolling it off the framework on a rod which constituted one edge of the frame. Neither soil nor air temperatures in the bench were affected appreciably by the cover.

The plants of the second bench in each house were given a light period of 17 to 19 hours by supplementing the natural day with artificial illumination from 4 P. M. until midnight. Three-hundred-watt Mazda-C bulbs in standard deep-bowl reflectors were suspended at 32-inch intervals, 4 feet above the level of the soil. The light intensity at the level of the soil was approximately 100 foot-candles. Thermographs directly beneath the lights recorded an increase in air temperature of 1° C. when the lights were on, but there was no appreciable effect on the soil temperature. As the plants increased in height, the lights were raised to 5 and eventually to 6 feet above the bench.

A third bench, adjacent to the one directly under the lights, received the same period of illumination but the intensity of the supplementary light was only 50 foot-candles at the edge of the bench nearest the lights, and 15 foot-candles in the corners farthest away.

The natural-day control plants of the fourth bench in each house were protected from the lights over the long-day bench by a heavy black sateen curtain hung in the intervening aisle during the period when the lights were on. The natural length of day increased from 9.5 hours at the beginning of the experiment to 15 hours when the last plants were harvested.

In order to determine whether liming would affect the growth and chemical composition of the plants, each bench was divided into two parts by a tight board partition, and commercial hydrated lime was mixed into the soil of the north section at the rate of 4 tons per acre. The reaction of 2:1 water extracts of the limed soil was pH 8.1 as compared with pH 7.0 for the untreated soil.

2. Sampling procedure

Each lot of plants was sampled in the early tillering stage and again at heading time. At the time of the first samplings, the plants were from 4 to 7 weeks old and had no stem tissue, so the material taken for analysis consisted of the entire plants, excepting the roots. The plants were always cut in the morning to avoid the effects of diurnal variations in composition. For the carbohydrate analyses the leaves were immediately ground to a pulp in a Nixtamal mill. Fifty-gram samples were quickly weighed into tared wide-mouthed 500-cc. Erlenmeyer flasks, each containing 0.2 gm. calcium carbonate. The pulp was then covered with enough boiling 95 per cent. alcohol to make the final concentration approximately 80 per cent., and boiled in a water bath for 15 minutes. Similar material was dried for 24 hours in a well ventilated steam-heated oven (55°–60° C.) for nitrogen determinations. A third lot of the leaves was ground through the mill and the juice squeezed through cheese-cloth by hand for pH determinations.

At the time of the second samplings, when the plants were in the heading stage, the leaves were stripped from the culms and those which were dried

or yellow were discarded. In all the samplings of both young and old plants, only green leaves were used. The culms, together with considerable leaf-sheath tissue, were analyzed separately.

At the time of the first samplings the plants of each group, *i.e.*, those subjected to the same temperature and soil reaction but to four different light conditions, were all cut on the same day in order to obtain close comparisons of day-length effects uncomplicated by the uncontrollable factors that differ from day to day. For the second samplings the plants of each lot were cut when the first flowers emerged, on the dates indicated in the tables.

3. Chemical analyses

Carbohydrates.—Reducing sugar, sucrose, and total sugar in the samples preserved in alcohol were extracted according to the following procedure. The original alcohol was decanted through a weighed filter paper into a 1000-cc. volumetric flask. The tissue was then extracted three times with 100-cc. portions of boiling 85 per cent. alcohol. On cooling, a gelatinous precipitate appeared, so the extractions were filtered into the original extract after standing overnight. The volumetric flask was then made up to volume with 85 per cent. alcohol and aliquots were removed into beakers. These aliquots were freed of alcohol by evaporating almost to dryness in a water bath at 60° with a strong current of air. They were then taken up in hot distilled water and cooled after a few minutes by the addition of more cold water. The solutions were cleared with neutral lead acetate and the excess lead precipitated with potassium oxalate.

The reducing values of the cleared solutions were determined by the Shaffer-Hartmann method (**44**), modified slightly with respect to the volumes of solutions used. From previous work on similar material it had been found that sucrose was the only dissacharide present in appreciable amounts. So both sucrose and total sugar were obtained by the hydrochloric acid inversion method of Herzfeld, as given by Browne (**6**, p. 266), and calculated from the reducing values by means of the Munson-Walker sugar tables.

After extraction of the sugars, the residue of each sample was transferred with the filter paper to an aluminum weighing can and weighed after drying for 48 hours at 100° C. The weight of the alcohol-soluble material was obtained by drying aliquots of the extracts on quartz sand in tared containers at 60° C. in a vacuum oven for 8 hours, after previous reduction to air dryness in a current of warm air. The weight of this alcohol-soluble material plus the weight of the extracted and dried residue constituted the total dry weight on which calculations of the sugars were based.

The acid-hydrolyzable material was determined after grinding the dried, extracted residue in an ordinary drug mill, then in a ball mill, until it

passed through an 80-mesh sieve. Two-gram samples, previously freed of water by drying at 100° C., were extracted with 2.5 per cent. H_2SO_4 for 2 hours on a sand bath. After hydrolysis the samples were filtered, neutralized, cleared with neutral lead acetate, and the excess lead removed with potassium oxalate. From the reducing power of aliquots of the cleared solutions, made to volume, the percentages of acid-hydrolyzable material were calculated as glucose.

Sufficient material for starch determinations was available at the heading stage only. One-gram samples of the original residue after extraction of the sugars were digested with a preparation of diastase known as Pangestin, a product of the Digestive Ferments Co. of Detroit, Michigan. After heating to boiling, and filtering, the solutions were made up to a concentration of 2.5 per cent. H_2SO_4 and hydrolyzed for 1 hour by boiling gently on a sand bath. After neutralizing and making to volume, reducing power was determined and the results calculated as percentages of starch.

All analytical procedures were carried out in duplicate. In the case of the glucose determinations, the maximum deviations from the mean of the two duplicates never exceeded an absolute difference of 0.1 per cent., so that differences greater than this in the glucose percentages given in the tables are probably real differences between the samples and not errors due to the method. Owing to the larger percentages of sucrose and total sugars present, their absolute percentage deviations from the mean of the duplicates increased to as much as 0.2, only 5 per cent. of the duplicates showing a greater discrepancy than this. It is therefore fairly safe to assume that, in this material, an absolute difference greater than 0.2 per cent. indicates a real difference in the samples. In the case of the acid-hydrolyzable data, the corresponding limit of accuracy is increased to 0.3, differences of this magnitude or less being obtained in 95 per cent. of the duplicate determinations.

NITROGEN.—Nitrogen, not including nitrates, was determined by the Kjeldahl method on material dried at 55°–60° C., then ground in a Wiley mill. The moisture content of the air-dry material was determined by drying duplicate portions to constant weight (48 hours) in an electric oven. The nitrogen determinations were thus corrected for the moisture content of the samples and expressed as percentages of the oven-dry weights.

The nitrogen values given in the tables are the averages of at least three replications. The maximum deviation from the mean of the three percentages was less than 0.1 in 98 per cent. of the samples, so differences greater than 0.1 per cent. between determinations on different samples are assumed to be significant.

ACIDITY.—Electrometric determinations of the pH values of the juice of the fresh tissues, cut within an hour after the material for the corre-

sponding carbohydrate and nitrogen samples, were made with a Wolf potentiometer and accessory equipment. The system was accurate to within ± 0.01 pH, according to frequent checks with M/20 potassium acid phthalate.

4. Calculations

The difficulty of expressing the analytical data without the distortion inevitably resulting when the dry-weight basis changes during the experiment seems insurmountable. Unless the data are expressed in terms of the absolute weight of material per plant or organ (which in the case of such variable plants as wheat (9) requires a large amount of material) they are merely statements of ratios of the constituent under consideration to the total solids, and there is a tendency to interpret changes in the ratio as changes in the numerator of the fraction only. Chibnall (8) pointed out that estimation of the diurnal changes in nitrogen from percentages based on the dry weight of the leaf gives inaccurate and misleading results, and recommended that such percentages be calculated on the less variable fresh weight of the tissue. Mason and Maskell (37) concluded that the apparent constancy of the fresh-weight basis is largely illusory and that in the absence of specific evidence of constancy, or especially when differential treatment might be expected to affect the moisture relations of the plant, this basis, like the customary dry-weight basis, is unsatisfactory. They suggest that when it can be safely assumed that the variation in the basic dry weight during the experiment is due to variation in labile carbohydrates, a logical procedure is to base analyses on the so-called residual dry weight, *i.e.*, the total dry weight minus these carbohydrates. Changes in nitrogen or ash constituents are assumed to be proportionately too small to produce appreciable effects on the data. The applicability of the method is limited to experiments involving mature tissues and extending over short periods of time during which the residual dry weight can be assumed to be practically constant (36).

In the present investigation, use of the fresh-weight basis was unsatisfactory because of uncertainty as to the constancy of the water relations under the different experimental conditions, and also because of the small magnitudes of some of the percentages. The use of the residual dry weight, the most logical in some respects, was precluded by the fact that the experiments were of long duration and involved rapidly growing plants, with every likelihood of variability of constituents other than labile carbohydrates under the different experimental conditions (36, 37). The total dry weight was also unsatisfactory because of its obvious inconstancy. Nevertheless, in the absence of a better method, the data are reported on this basis, which has the advantage of being the one most commonly used. It introduces magnifying effects in some instances and compensating effects in

others, but the trends shown by the data as calculated by all three methods are, in general, the same. In table II and III, where a discrepancy in the trend of nitrogen with temperature appeared which seemed related to a concomitant variation in the dry weights, the results as calculated by both residual and total dry weights are reported.

The symbol C-h/N is used in the tables to denote the ratio between the total carbohydrates and the nitrogen percentages.

Growth responses

The control plants with the natural length of day produced the best growth and yield in both the spring and the winter varieties. The plants all grew more vigorously at the low (10°–12°) than at the high (21°–23°) temperature. Thus the best plants of both varieties were the natural-day control plants of the low-temperature house. The degree of injury produced by the unfavorable factors—the lengthened light period, the shortened light period, and the high temperature—differed markedly in the two varieties

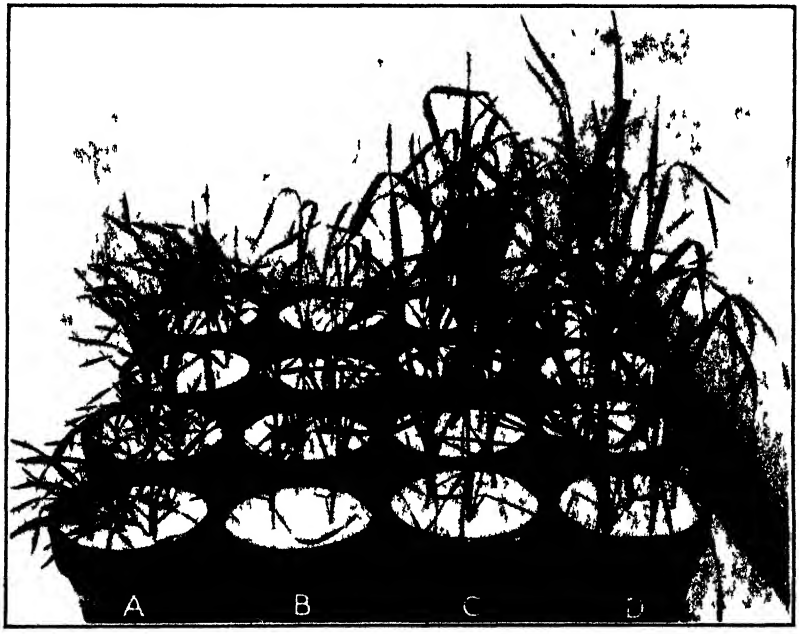


FIG. 1. Hard Federation wheat sown Feb. 25, 1930, photographed at age of 1 month (21°–23° C.):

A, short (8-hour) day
B, natural (12-hour) day
C, long (18-hour) day; supplementary light, 15–50 foot-candles
D, long (18-hour) day; supplementary light, 100 foot-candles.

The long daily light period forced the plants of the spring variety, Hard Federation, into heading so rapidly that at both temperatures there was insufficient vegetative development for normal head and grain formation. The plants were short and stunted in appearance at maturity, with reduced tillering and with small leaves and heads. Plants of Turkey, the winter variety growing beside them, were less sensitive to the forcing action of the long day, and, at the low temperature, appeared normal except for a reduction in number of tillers. In fact, their average weights of grain per head and their average kernel weights exceeded those of the control plants (table I), but their smaller number of tillers lowered their total yield.

TABLE I

EFFECTS OF ENVIRONMENTAL FACTORS ON HEAD AND KERNEL DEVELOPMENT OF HARD FEDERATION AND TURKEY WHEAT, 1930*

DAY LENGTH	SOIL REACTION	HARD FEDERATION		TURKEY	
		10°–12° C.	21°–23° C.	10°–12° C.	21°–23° C.
		Weight of grain from 100 heads			
	pH	*gm.*	*gm.*	*gm.*	
Short (8 hours)	7.0	78.51	40.46	0	n†
	8.1	74.65	34.42	0	n
Natural (9.5–15 hours)	7.0	117.40	88.30	104.40	f
	8.1	135.59	101.89	96.09	f
Long (18 hours)	7.0	3.07	20.68	118.80	f
(15–50 foot-candles)	8.1	3.48	16.94	121.73	f
Long (18 hours)	7.0	7.85	22.85	120.34	f
(100 foot-candles)	8.1	6.73	.....	120.69	f
		Weight of 100 kernels			
Short (8 hours)	7.0	3.40	2.96	.....	.....
	8.1	3.63	2.95	.....	.....
Natural (9.5–15 hours)	7.0	4.92	4.08	3.78	.....
	8.1	4.91	4.02	3.31	.....
Long (18 hours)	7.0	4.51	3.24	4.33	.....
(15–50 foot-candles)	8.1	4.39	3.14	4.12	.....
Long (18 hours)	7.0	4.77	3.33	4.27	.....
(100 foot-candles)	8.1	4.85	.....	4.26	.....

* Temperatures were maintained during first four months only.

†n, no heads formed; f, few heads formed, mostly sterile.

At the low temperature, the short daily light period produced large vegetative plants in both varieties, with increased tillering and leaf development and retarded heading and maturation, as compared with the controls with the natural day. The winter variety was more injured than was the spring variety at this day length, its heads being completely sterile.

At the high temperature, heading in Turkey was almost completely inhibited at every day length, whereas Hard Federation produced almost as much grain at this temperature as at the lower one, although the plants were all shorter.

The extreme susceptibility of Hard Federation to the forcing action of the long day is shown in figures 1 and 2. Owing to the difficulty of

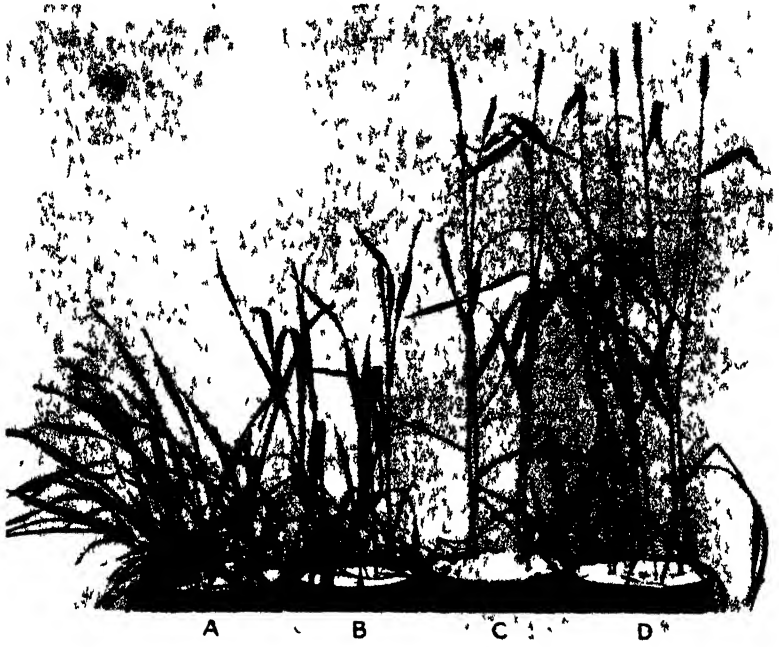


FIG. 2. Hard Federation wheat sown Feb. 25, 1930, photographed at age of 2 months (21°–23° C):

A, short (8-hour) day
B, natural (13-hour) day
C, long (18-hour) day; supplementary light, 15–50 foot-candles
D, long (18 hour) day, supplementary light, 100 foot-candles.

photographing the plants in the benches, those shown in these figures were grown in pots for the pictures, the soil being covered with a layer of white sand. In figure 1 is shown the rapid development of the long-day plants and the increased tillering of the short-day plants. In figure 2 some of these plants are shown a month later when those of the long day were past flowering. The control plants (*B*) with the natural day were in an early shooting stage, and the short-day plants (*A*) were still in the tillering stage. Subsequently the height of both the short- and the natural-day plants greatly surpassed that of the long-day plants (*C*, *D*).

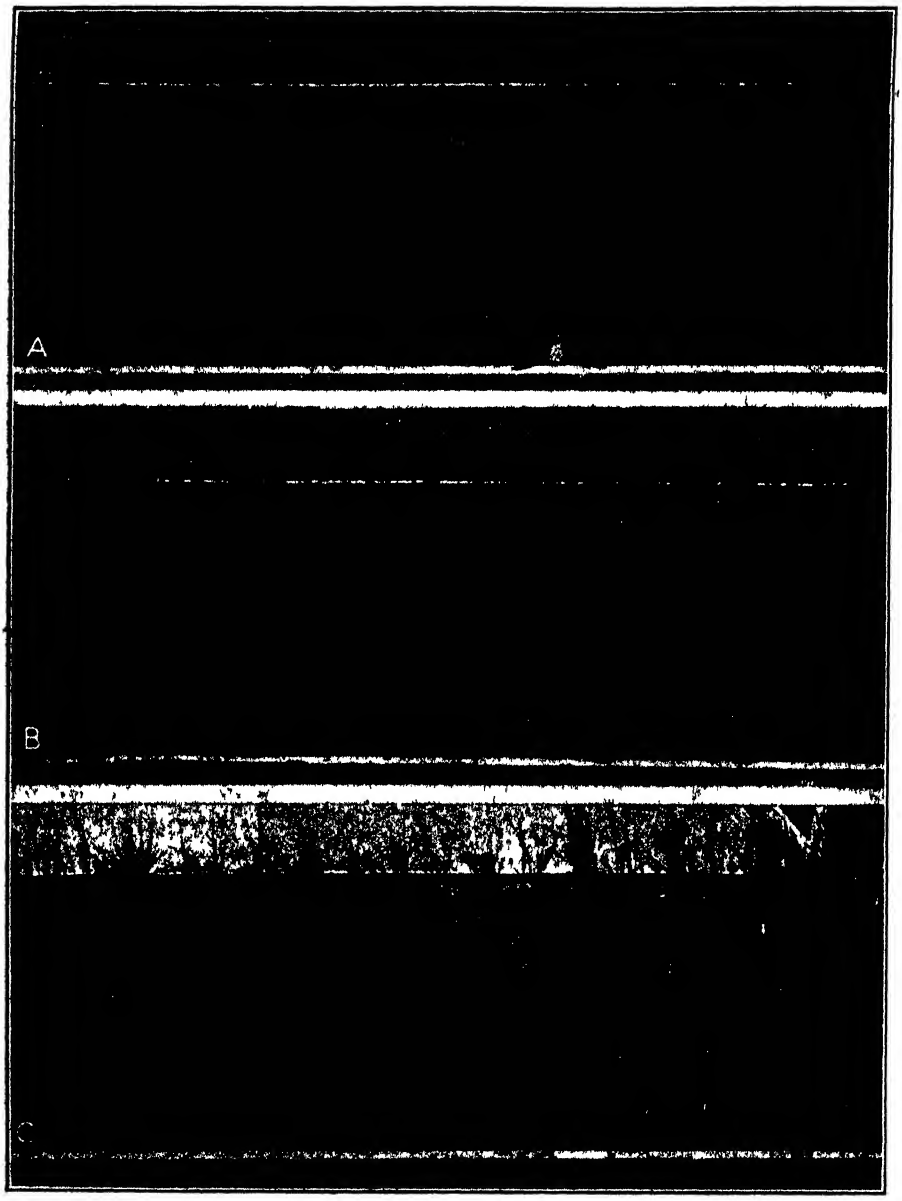


FIG. 3. Turkey (left) and Hard Federation (right), sown Dec. 5, 1929, photographed at age of 1 month (10°–12° C.):
A, short (8-hour) day
B, natural (9.5-hour) day
C, long (16.5-hour) day.

A conspicuous modification of growth habit induced by the long day was the inhibition of the recumbent type of growth, normally characteristic of fall sowings of Turkey and similar varieties during the winter months (**28**). Only at the shorter day lengths, *i.e.*, the artificial one of 8 hours and the natural winter day of about 10 hours, did the plants become recumbent. The resulting difference in the appearance of the plants near the time of the first samplings is shown in figure 3. At the short and at the natural days the Turkey plants are shown in the prostrate condition with the erect plants of Hard Federation beside them (*A*, *B*). At the long day (*C*) Turkey was as erect as Hard Federation, never having passed through a prostrate stage.

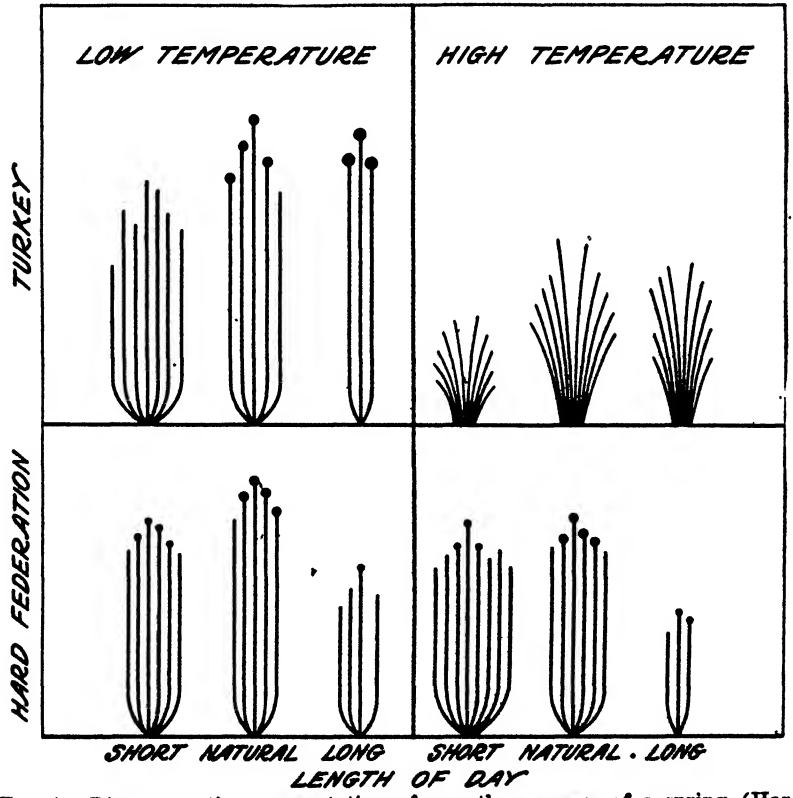


FIG. 4. Diagrammatic representation of growth responses of a spring (Hard Federation) and winter (Turkey) wheat to short (8-hour), natural (9.5 to 15-hour), and long (17-hour) days at low (12° ± 1°) and high (21° ± 1°) temperatures. Heights are drawn to scale from averages. The number of verticals with dots shows the average number of fertile tillers per plant, and those without dots, the sterile tillers. Relative yield per head is roughly shown by small, medium, and large dots on the verticals. Leafy sterile plants of Turkey with few or no culms are represented by groups of spreading lines.

The increase in soil alkalinity from pH 7.0 to pH 8.1 in the limed end of each bench had no appreciable effect on the growth or rate of development of the plants of either variety. The difference in the intensity of the supplementary illumination prevailing in the two long-day benches of each house also produced no visible differences in the plants.

A general characterization of the type of growth of each lot of plants is given in the tables beside the corresponding chemical data. Data on head and kernel development are recorded in table I. Average growth measurements for similar plants grown under approximately the same conditions are represented diagrammatically in figure 4, which shows the relative height, number of fertile and sterile tillers, and relative yield of grain of both varieties under the six different conditions of day length and temperature. Detailed descriptions of these plants are reported elsewhere (**29**).

Results of chemical analyses

1. Effects of day length on carbohydrates, nitrogen, and pH values of wheat leaves

Young plants.—The data in tables II and III show that in young plants of both varieties there was generally a positive correlation between the amount of carbohydrates in the tissues and the length of the daily period of illumination. The glucose fraction varied directly with day length without exception in Turkey (table II), but with less consistency in some groups of Hard Federation (table III). The data on sucrose were not consistently correlated with day length in either variety. The figures for total sugar, acid-hydrolyzable material, and total carbohydrates leave no doubt but that the long day tended to bring about an increase in these compounds and that the short day tended to decrease them. The accumulation of sugar brought about by the 17-hour day was especially great in the low-temperature plants of Turkey (groups 1 and 3 of table II).

The effect of day length on the percentage of nitrogen in the tissues was the opposite of the effect on carbohydrates. Without exception the short light period increased it and the long one decreased it, in comparison with the control plants with the natural day. That the low nitrogen content of the long-day plants might have been responsible for their type of growth is suggested by the similarity between some of their abnormalities—such as reduced tillering, decreased vegetative growth, hastened maturation, and, in Turkey, increased weight of individual kernels—and the abnormalities caused by deficient nitrogen supply (**11**).

The data obtained by Garner, Bacon, and Allard (**18**), showing that shortening the day increases the sugar content of cosmos (which flowers with a short day) while lengthening the day increases the sugar content

of radish (which flowers with a long day), suggest that the effects of day length on carbohydrates vary with the photoperiodic characteristics of the species, or variety, thus offering a possible explanation for some of the conflicting reports in the literature on this subject. Wheat is a long-day plant. The evidence from the present study and from that of ARTHUR, GUTHRIE, and NEWELL (1), pointing to a direct relationship between day length and carbohydrate content in wheat, is therefore consistent with this generalization.

The pH values given in tables II and III show that in Hard Federation at the low temperature (groups 5, 7) there were no consistent effects of day length on acidity; but at the high temperature, and, in Turkey, at both temperatures, the shortened light period generally increased and the lengthened one decreased the pH value of the juice. This relation is consistent with GARNER, BACON, and ALLARD'S report (18) that, in long-day plants, exposure to a short light period results in the acidity remaining at a low level, while exposure to a long one commonly causes it to increase.

HEADING PLANTS.—In tables IV and V are given the results of analyses for each lot of plants when they reached the heading stage, or, in the case of those which did not head, an advanced age. The plants of the different day lengths constituting each group could not be sampled on the same day, as they had been at the earlier samplings, because of their widely different rates of development. Each lot of plants was therefore sampled when the majority of the heads had emerged from the boot, with the earliest ones flowering.

Environmental conditions had changed considerably for these older plants. The controls, subjected to the natural light period, were receiving from 13 to 15 hours of light per day, depending on the date of cutting, instead of the 10 hours or less that they had received in the winter months. Temperature control had to be discontinued the second week of April, by which time only the most rapidly developing plants of Hard Federation had reached the heading stage. The rest were therefore subjected to considerably higher temperatures for various periods, depending on their dates of heading. The approximately 10° difference between the low- and high-temperature houses, maintained during the first four months, was reduced to 5°. The light intensity had of course increased during the spring months. The environments were thus quite different from those responsible for the results in tables II and III.

Unlike the data on the younger plants, those obtained at heading time did not show the regular trends of increasing carbohydrates and decreasing nitrogen percentages with increasing day length. In Turkey (table IV) there was still a definitely higher total sugar and total carbohydrate concentration in the leaves of the plants with the long day, but this relation no

TABLE II

EFFECTS OF ENVIRONMENTAL FACTORS ON CHEMICAL COMPOSITION OF YOUNG PLANTS OF TURKEY, A WINTER WHEAT
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS WHEN NOT INDICATED OTHERWISE]

DAY LENGTH*	DATE OF SAMPLING (1930)	DRY WEIGHT (PER CENT OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	ACID HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN BASED ON		C-h/N	pH OF JUICE	SUBSEQUENT DEVELOPMENT
		TOTAL	RESIDUAL						TOTAL DRY WT.	RESIDUAL DRY WT.			
		Group 1: plants grown at 10°–12° C., neutral soil											
		%	%	%	%	%	%	%	%	%			
Short (8)	2/1	17.82	14.49	2.32	5.71	8.05	10.66	18.71	6.21	7.64	3.01	6.12	Vegetative; sterile heads
Natural (10)	2/1	17.92	14.25	2.79	6.01	8.85	11.64	20.49	5.42	6.82	3.78	6.04	Normal; good yield
Long (17)†	2/1	17.57	12.78	4.46	9.26	13.85	13.43	27.28	4.57	6.29	5.97	5.96	Almost normal; good yield
Long (17)‡	2/1	17.55	12.86	4.49	8.82	13.43	13.28	26.71	4.46	6.09	5.99	5.96	Almost normal; good yield
		Group 2: plants grown at 21°–23° C., neutral soil											
Short (8)	1/10	12.42	10.84	1.12	1.75	2.83	10.14	12.77	5.56	6.38	2.30	5.99	Leafy growth; sterile
Natural (10)	1/10	12.97	11.02	1.63	2.50	4.09	10.94	15.03	5.29	6.22	2.84	5.88	Leafy growth; almost sterile
Long (17)†	1/10	11.87	9.95	2.28	2.11	4.33	11.85	16.18	4.50	5.37	3.60	5.86	Leafy growth; almost sterile
Long (17)‡	1/10	13.21	10.98	2.16	2.21	4.34	12.61	16.95	4.60	5.53	3.68	5.82	Leafy growth; almost sterile

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE II.—(*Continued*)

Day length*	Date of sampling (1930)	Dry weight (per cent of wet weight)		Glucose	Sucrose	Total sugar	Acid hydrolyzable material including starch	Total carbohydrates	Nitrogen based on		C-h/N	pH of juice	Subsequent development
		Total	Residual						Total dry wt.	Residual dry wt.			
		Group 3: plants grown at 10°–12° C., alkaline soil											
		%	%	%	%	%	%	%	%	%			
Short (8)	1/25	16.97	13.56	2.43	7.36	9.86	10.24	20.10	5.39	6.75	3.73	6.07	Vegetative; sterile heads
Natural (10)		16.87	13.30	2.64	7.20	9.87	11.30	21.17	5.14	6.52	4.12	5.98	Normal; good yield
Long (17)†		17.06	12.91	3.59	8.78	12.44	11.91	24.35	4.61	6.10	5.28	6.00	Almost normal; good yield
Long (17)‡		16.98	12.63	3.99	9.22	13.40	12.23	25.63	4.48	6.02	5.72	5.95	Almost normal; good yield
		Group 4: plants grown at 21°–23° C., alkaline soil											
Short (8)	1/11	11.84	10.26	0.82	0.80	1.58	11.81	13.39	5.67	6.55	2.36	6.10	Leafy growth; sterile
Natural (10)		11.96	10.44	1.08	0.36	1.39	11.31	12.70	5.22	5.99	2.43	6.05	Leafy growth; almost sterile
Long (17)†		11.52	9.77	1.58	0.83	2.32	12.91	15.23	4.90	5.78	3.11	5.96	Leafy growth; almost sterile
Long (17)‡		11.90	9.98	1.48	0.84	2.20	13.98	16.18	4.86	5.80	3.33	5.94	Leafy growth; almost sterile

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE III

EFFECTS OF ENVIRONMENTAL FACTORS ON CHEMICAL COMPOSITION OF YOUNG PLANTS OF HARD FEDERATION, A SPRING WHEAT
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS WHEN NOT INDICATED OTHERWISE]

DAY LENGTH*	DATE OF SAMPLING (1930)	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN BASED ON		C-h/N	pH OF JUICE	SUBSEQUENT DEVELOPMENT
		TOTAL	RESIDUAL						TOTAL DRY WT.	RESIDUAL DRY WT.			
		Group 5: plants grown at 10°–12° C., neutral soil											
		%	%	%	%	%	%	%	%	%			
Short (8)	1/29	13.67	11.59	1.22	2.66	3.85	11.37	15.22	6.30	7.43	2.42	6.08	Vegetative; reduced yield
Natural (10)	1/29	14.27	12.01	1.50	3.79	5.28	10.58	15.86	5.95	7.07	2.67	6.03	Normal; good yield
Long (17)†	1/29	13.94	11.30	1.89	3.88	5.79	13.16	18.95	5.34	6.59	3.55	6.08	Stunted; poor yield
Long (17)‡	1/29	14.80	11.88	1.71	3.80	5.54	14.20	19.74	5.04	6.28	3.92	6.07	Stunted; poor yield
		Group 6: plants grown at 21°–23° C., neutral soil											
Short (8)	1/9	11.86	10.00	2.11	2.52	4.55	11.63	15.71	5.56	6.64	2.83	5.98	Vegetative; poor yield
Natural (10)	1/9	11.77	9.96	1.42	2.02	3.47	11.85	15.32	5.21	6.15	2.94	5.93	Fair growth; moderate yield
Long (17)†	1/9	11.52	9.52	2.34	1.92	4.40	12.95	17.35	4.72	5.72	3.68	5.87	Stunted; poor yield
Long (17)‡	1/9	11.79	9.60	1.84	2.12	3.95	14.65	18.56	4.58	5.63	4.05	5.87	Stunted; poor yield

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE III.—(*Continued*)

Day length*	Date of sampling (1930)	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen based on		C-h/N	pH of juice	Subsequent development
		Total	Residual						Total dry wt.	Residual dry wt.			
		Group 7: plants grown at 10°–12° C., alkaline soil											
		%	%	%	%	%	%	%	%	%			
Short (8)	1/28	13.98	12.04	0.87	0.72	1.62	12.26	13.88	6.51	7.56	2.13	6.09	Vegetative; reduced yield
Natural (10)		13.63	11.63	1.29	1.02	2.25	12.50	14.75	6.31	7.40	2.34	6.10	Normal; good yield
Long (17)†		13.82	11.47	1.20	1.28	2.44	14.57	17.01	5.38	6.47	3.16	6.08	Stunted; poor yield
Long (17)‡		13.26	10.84	1.33	1.11	2.36	15.92	18.28	5.25	6.43	3.48	6.11	Stunted; poor yield
		Group 8: plants grown at 21°–23° C., alkaline soil											
Short (8)	1/13	11.29	9.82	1.05	0.35	1.35	11.73	13.08	5.84	6.72	2.24	5.94	Vegetative; poor yield
Natural (10)		11.71	10.02	1.55	0.79	2.29	12.11	14.40	5.66	6.61	2.54	5.95	Fair growth; moderate yield
Long (17)†		11.67	9.80	1.68	0.80	2.44	13.60	16.04	4.98	5.93	3.22	5.90	Stunted; poor yield
Long (17)‡		11.22	9.37	1.75	1.10	2.82	13.70	16.52	4.78	5.73	3.46	5.87	Stunted; poor yield

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE IV

CHEMICAL COMPOSITION OF LEAVES AND CULMS OF TURKEY WHEAT IN THE HEADING STAGE, GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

[CARBOHYDRATES AND NITROGEN EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH*	DATE OF SAMPLING (1930)	TISSUE	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	STARCH	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C-h/N	pH OF JUICE	CONDITION OF PLANTS
			TOTAL	RESIDUAL										
	Group 1: low temperature, neutral soil													
			%	%	%	%	%	%	%	%	%			
Short (8)	6/26	Leaves	28.50	22.38	1.75	4.40	6.20	1.42	15.25	21.45	3.66	5.86	5.62	Poor; sterile heads
		Culms	27.12	17.67	9.03	7.28	16.33	2.26	18.53	34.86	1.46	23.9	5.87	
Natural (14)	5/15	Leaves	23.58	18.70	1.02	3.14	4.22	1.58	16.57	20.76	3.23	6.43	5.78	Normal; high yield
		Culms	22.36	15.87	3.40	3.31	6.68	2.08	22.38	29.06	0.74	39.3	5.83	
Long (19)†	4/25	Leaves	23.78	17.95	1.61	7.49	9.21	2.55	15.32	24.50	3.84	6.38	5.90	Good; high yield
		Culms	27.27	17.20	6.12	12.71	18.90	2.81	18.05	36.95	1.18	31.3	5.83	
Long (19)‡	4/26	Leaves	24.78	18.63	1.86	7.45	9.44	2.43	15.36	24.71	3.63	6.81	5.97	Good; high yield
		Culms	27.62	17.50	5.43	13.12	18.64	2.80	18.03	36.67	1.00	36.7	5.94	
	Group 2: high temperature, neutral soil													
Short (8)	6/19	Leaves	18.60	15.52	1.33	2.73	4.04	1.56	12.50	16.54	4.09	4.04	5.66	Poorly vegetative; no heads
Natural (15)	6/19	Leaves	20.71	16.54	2.80	2.30	5.12	2.13	15.00	20.12	3.05	6.60	5.52	Poorly vegetative; few heads
Long (19)†	6/19	Leaves	24.00	19.16	3.32	2.11	5.44	2.00	14.71	20.15	3.24	6.22	5.41	Poorly vegetative; few heads
Long (19)‡	6/19	Leaves	23.88	18.56	3.95	2.83	6.70	2.20	15.61	22.31	3.47	6.43	5.41	Poorly vegetative; few heads

* Numbers in parentheses indicate hours.

† 15–50 foot-candles.

‡ 100 foot-candles.

TABLE IV—(*Continued*)

Day length*	Date of sampling (1930)	Tissue	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Starch	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen	C-h/N	pH of juice	Condition of plants
			Total	Residual										
	Group 3: low temperature, alkaline soil													
			%	%	%	%	%	%	%	%	%			
Short (8)	6/26	Leaves	29.70	23.37	1.44	3.56	5.03	0.88	16.30	21.33	3.45	6.18	5.60	Poor; sterile heads
		Culms	27.18	17.49	8.35	8.37	16.75	2.19	18.93	35.68	1.57	22.7	5.82	
Natural (14)	5/15	Leaves	23.28	18.40	1.73	3.72	5.48	1.62	15.47	20.95	3.44	6.09	5.65	Normal; high yield
		Culms	26.84	17.89	5.84	5.84	11.70	2.27	21.64	33.40	0.85	39.3	5.67	
Long (19)†	4/24	Leaves	26.46	19.26	2.07	9.25	11.39	2.69	15.82	27.11	3.65	7.43	5.82	Good; high yield
		Culms	29.34	17.95	6.32	13.31	19.81	3.17	19.00	38.81	0.99	39.2	5.87	
Long (19)‡	4/23	Leaves	24.88	18.38	2.12	7.90	10.07	2.77	16.06	26.13	3.58	7.30	5.88	Good; high yield
		Culms	27.84	17.91	7.57	8.71	16.31	2.77	19.34	35.65	0.95	37.5	5.81	
	Group 4: high temperature, alkaline soil													
Short (8)	6/19	Leaves	18.45	15.25	1.70	2.64	4.34	2.32	13.04	17.38	4.25	4.09	5.59	Poorly vegetative; no heads
Natural (15)	6/19	Leaves	24.08	19.31	2.37	2.21	4.57	2.04	15.25	19.82	3.09	6.41	5.32	Poorly vegetative; few heads
Long (19)†	6/19	Leaves	23.35	18.84	3.17	2.31	5.46	1.71	13.84	19.29	3.41	5.66	5.40	Poorly vegetative; few heads
Long (19)‡	6/19	Leaves	23.05	18.43	3.21	2.36	5.58	1.51	14.45	20.03	3.25	6.16	5.31	Poorly vegetative; few heads

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE V

CHEMICAL COMPOSITION OF LEAVES AND CULMS OF HARD FEDERATION WHEAT IN THE HEADING STAGE, GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS
[CARBOHYDRATES AND NITROGEN EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH*	DATE OF SAMPLING (1930)	TISSUE	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	STARCH	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C-h/N	pH OF JUICE	CONDITION OF PLANTS
			TOTAL	RESIDUAL										
	Group 5: low temperature, neutral soil													
			%	%	%	%	%	%	%	%	%			
Short (8) ...	5/13	Leaves	21.44	16.89	1.01	2.42	3.42	1.69	17.86	21.28	3.48	6.11	5.95	Fair growth; reduced yield
		Culms	18.67	14.41	1.54	1.25	2.82	1.92	20.00	22.83	1.01	22.6	5.79	
Natural (13)	4/10	Leaves	22.36	17.07	1.91	5.65	7.63	2.18	16.09	23.67	3.61	6.56	5.99	Normal; good yield
		Culms	22.35	15.15	6.01	6.57	12.62	2.89	19.74	32.24	.89	36.2	5.79	
Long (18)† ...	3/3	Leaves	22.70	18.49	0.97	5.99	7.05	1.92	11.46	18.53	4.13	4.49	6.32	Stunted; very low yield
		Culms	22.41	15.67	3.22	7.52	10.77	2.68	19.32	30.09	1.45	20.8	6.25	
Long (18)‡ ...	3/1	Leaves	21.68	17.30	1.32	5.98	7.38	2.81	12.87	20.25	4.10	4.94	6.37	Stunted; very low yield
		Culms	23.66	16.38	3.83	8.09	11.95	2.40	18.83	30.78	1.55	19.9	6.20	
	Group 6: high temperature, neutral soil													
Short (8)	4/19	Leaves	21.40	17.17	0.73	1.99	2.71	1.50	17.07	19.78	3.52	5.62	6.01	Poor growth; low yield
		Culms	18.79	14.42	1.74	1.38	3.16	2.19	20.11	23.27	1.61	14.5	5.96	
Natural (13)	3/28	Leaves	22.82	17.54	1.91	6.21	8.20	1.59	14.97	23.17	3.54	6.55	6.03	Fair growth; moderate yield
		Culms	24.55	16.47	6.94	6.82	13.79	2.68	19.14	32.93	1.36	24.2	5.99	
Long (17)§ ...	1/31	Leaves	17.32	14.20	0.98	4.87	5.86	1.79	12.08	17.94	3.98	4.51	6.08	Stunted; poor yield
		Culms	17.81	13.57	2.25	1.32	3.48	2.10	20.32	23.80	2.10	11.3	.....	

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.
§ Long-day plants of both light intensities taken together to make up one sample.

TABLE V—(*Continued*)

Day length*	Date of sampling (1930)	Tissue	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Starch	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen	C-h/N	pH of juice	Condition of plants
			Total	Residual										
			Group 7: low temperature, alkaline soil											
			%	%	%	%	%	%	%	%	%			
Short (8)	5/13	Leaves	21.00	16.97	0.87	2.44	3.30	2.00	15.88	19.18	3.61	5.31	5.93	Fair growth; reduced yield
		Culms	17.08	12.95	2.07	1.19	3.28	1.57	20.93	24.21	1.29	18.8	5.80	
Natural (13)	4/15	Leaves	21.48	17.29	0.92	3.62	4.54	1.81	14.97	19.51	4.07	4.79	5.99	Normal; good yield
		Culms	20.25	14.91	4.90	1.84	6.65	2.24	19.72	26.37	1.27	20.7	6.00	
Long (18)† ..	3/3	Leaves	21.44	17.22	0.89	5.69	6.65	2.38	13.00	19.65	4.51	4.36	6.15	Stunted; very low yield
		Culms	22.72	16.30	3.19	6.63	9.80	2.70	18.48	28.28	1.73	16.3	6.23	
Long (18)‡...	3/1	Leaves	21.54	17.26	1.19	5.94	7.18	2.17	12.66	19.73	4.40	4.48	6.30	Stunted; very low yield
		Culms	23.40	16.52	3.93	6.88	10.77	2.58	18.65	29.42	1.72	17.1	6.20	
			Group 8: high temperature, alkaline soil											
Short (8)	4/21	Leaves	21.06	16.48	0.82	3.66	4.50	1.25	17.30	21.80	3.46	6.30	6.12	Poor growth; low yield
		Culms	19.01	14.40	1.93	2.10	3.93	2.25	20.40	24.33	1.63	14.9	6.00	
Natural (13)	4/2	Leaves	21.92	17.47	1.69	4.02	5.72	1.62	14.58	20.30	3.73	5.44	5.99	Fair growth; moderate yield
		Culms	21.35	15.36	4.94	4.50	9.37	2.15	18.72	28.09	1.46	19.2	6.10	
Long (17)§...	1/31	Leaves	17.48	14.44	0.80	4.85	5.68	.	11.69	17.37	4.54	3.83	6.08	Stunted; poor yield
		Culms	16.37	12.28	2.65	0.94	3.42	2.58	21.57	24.95	2.21	11.3	.....	

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.
§ Long-day plants of both light intensities taken together to make up one sample.

longer appeared consistently in the data for Hard Federation (table V). In the latter variety the leaves of the 8-hour-day plants still had the lowest sucrose and total sugar values in every group, but they had the highest percentages of acid-hydrolyzable carbohydrates. In fact, the acid-hydrolyzable fraction of the leaves of Hard Federation decreased as regularly with increasing day length in every group of the heading plants as it had increased in the case of the younger plants of table III.

The pH values of the leaf juice of the heading plants of tables IV and V were highest in the case of the rapidly maturing plants of the long day. This relation has no significance so far as day length effects are concerned, inasmuch as the long-day plants were much the youngest at the time of cutting. The pH value decreases with age during the maturation period (**24, 25, 26**). The tendency of the carbohydrates to increase and the nitrogen percentages to decrease with age may have similarly obscured the relations between these constituents and day length that had appeared so distinctly at the earlier growth stage.

A marked difference between the chemical composition of the leaves and culms is shown by the carbohydrate and nitrogen percentages given in tables IV and V. The culms of both varieties under all the different environmental conditions were characterized by relatively high carbohydrate and low nitrogen percentages. The glucose percentages were generally from two to three times as high in the culms as in the leaves. The percentages of acid-hydrolyzable material were also consistently higher in the culms. The nitrogen content of the culms was not over one-half and usually but one-third or less of the amount in the leaves. There was but little difference in the pH values.

OUTDOOR EXPERIMENTS.—Through the courtesy of Mr. H. A. ALLARD of the Bureau of Plant Industry, space was made available for growing some wheat plants under outdoor conditions identical with those obtaining in his day-length investigations (**16, 17**). Plants of Hard Federation were grown in deep flats placed on trucks which were pushed in and out of light-tight houses at stated hours each day so as to provide 8-, 10-, and 12-hour light periods. The control plants with the natural day, which increased from 12 to 14 hours during the experiment, were left in the open continuously. The plants from each condition were cut on May 9, 1930, in the tillering stage, at the age of 7 weeks. The culms were just beginning to elongate at the two shorter day lengths, and some had reached heights of as much as 6 cm. at the two longer ones. Analyses of the leaves are reported in table VI.

The data show the same increase in carbohydrates and decrease in nitrogen with increasing length of the daily light period as shown by the young greenhouse plants of tables II and III.

TABLE VI

EFFECTS OF DAY LENGTH ON CARBOHYDRATES, NITROGEN, AND pH VALUES OF THE LEAVES OF YOUNG, OUTDOOR HARD FEDERATION PLANTS SOWN MARCH 24, CUT MAY 9, 1930
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH	DRY WEIGHT (PER CENT. OF WET WEIGHT)	GLUCOSE	SUCROSE	TOTAL SUGAR	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C–h/N	pH OF JUICE
hrs.	%	%	%	%	%	%	%		
8	13.42	2.40	2.50	4.82	10.37	15.55	4.90	3.17	5.74
10	13.63	2.52	3.36	5.88	10.36	16.24	4.68	3.47	5.73
12	15.08	2.70	2.62	5.31	12.07	17.39	4.04	4.30	5.72
14 (control)	15.66	2.94	2.98	5.92	13.13	19.05	3.97	4.80	5.69

The carbohydrate percentages in table VI are of the same order of magnitude as those shown by table III to characterize the young greenhouse plants of this variety. The nitrogen percentages and the pH values were lower than in the greenhouse plants. Data of other experiments also have shown a general tendency toward lower nitrogen and pH values, and higher dry weights and sugar percentages, in field plants as compared with greenhouse plants, although the differences are not large.

2. EFFECTS OF INTENSITY OF SUPPLEMENTARY LIGHT

The appearance and rate of development of the plants immediately under the lights supplying the supplementary illumination for the long-day condition, where the light intensity was approximately 100 foot-candles, did not differ from that of the plants in the adjacent bench in which the light intensity was only 15 to 50 foot-candles. Height, tillering, dates of heading, and maturation of both varieties were alike in the two benches. On analysis, however, it was found that there was a small increase in total carbohydrates in the plants with the more intense supplementary illumination in all but one of the eight groups in tables II and III. None of the sugar fractions showed this relation consistently. Thus the increase was largely due to increased percentages of the acid-hydrolyzable material.

The percentage of nitrogen in the long-day plants with the higher supplementary light intensity was generally slightly lower than in those with the lower intensity. There were no differences attributable to this factor in the pH values.

In the older plants of tables IV and V there were no consistent differences in any measurement attributable to the difference in light intensity.

3. Effects of soil reaction

In all but the low-temperature groups of Turkey (groups 1 and 3), the data for both varieties in tables II and III showed markedly lower carbohydrate percentages in the plants of the more alkaline soil (pH 8.1) than in those of corresponding day lengths and temperatures from the neutral soil (pH 7.0). The total sugar percentages for the unlimed plants were approximately twice those of corresponding plants from the limed soil. The difference was mostly due to a reduction in sucrose in the limed plants, although there was an appreciable reduction in glucose also. Bystrikov (7) found that increases in soil alkalinity between pH 5.7 and 7.8 brought about progressive decreases in monosaccharides, although his total sugar percentages were not affected.

The acid-hydrolyzable material was not reduced by liming. In fact, there was frequently a slight increase in this fraction in the plants of those groups whose sugar contents were reduced, thus accounting for the proportionately lesser reductions in the total carbohydrate figures than in the sugar percentages.

The young plants of the limed soil with the low sugar percentages generally had somewhat higher nitrogen percentages. These data, including those of the low-temperature plants of Turkey which showed neither the decrease in sugar nor the increase in nitrogen with the limed soil, constitute an illustration of the frequently found inverse relation between the carbohydrate and nitrogen percentages.

The data for the older plants (tables IV and V) showed no consistent effects of soil reaction on these constituents.

There were no consistent differences in the pH values for corresponding plants from the limed and unlimed soils, either in the case of the young plants (tables II and III) or in those that were heading (tables IV and V). In some groups (table III) the values for corresponding plants from the limed and unlimed ends of the benches were alike within a few hundredths of a pH unit. It has been suggested previously (24) that liming the soil probably increases the pH value only when it favorably affects the growth and vigor of the plant. In the present experiment the lime had no visible effect on growth or rate of development, so an absence of effect on the pH value might therefore be expected.

4. Effects of temperature

Young plants.—Table II shows a relatively enormous accumulation of sugar at the low temperature in the plants of the winter variety, at all day

lengths. In the neutral soil the low-temperature plants had from two to more than three times as much total sugar, and those in the limed soil six to eight times as much, as did the corresponding plants grown at temperatures about 10° higher. Both glucose and sucrose were higher in the low-temperature plants, but the difference in sucrose was the more extreme. The acid-hydrolyzable fraction did not differ appreciably at the two temperatures. Evidently the hemicellulose, which constitutes the largest part of this fraction, is more easily affected by day length than by temperature.

The low-temperature plants of the spring variety (table III) did not have the high carbohydrate contents so characteristic of the winter variety growing beside them. In fact, there was no significant difference between the low- and high-temperature plants of this variety with respect to any of the carbohydrate fractions. Thus it would seem that the carbohydrate metabolism of Turkey was more affected by both temperature and day length than was that of Hard Federation.

In Hard Federation (table III) the percentage of nitrogen was less at the high than at the low temperature in every group. In Turkey (table II) this relation did not always hold for percentages based on the total dry weight of the tissues, because the large accumulation of carbohydrates, especially in the long-day plants, resulted in disproportionately high total dry weights for the plants of the low-temperature groups of this variety (groups 1 and 3). While this difference in the dry weights merely reduced the contrast in the carbohydrate percentages at the two temperatures, in some groups it completely obscured the usual difference in nitrogen found in preceding experiments—and in the other variety of the present experiment (table III)—in which there were no large accumulations of carbohydrates at the low temperature. When the inequalities in the dry weights were partially corrected by calculating the data on the residual dry-weight basis, the usual inverse correlation of nitrogen with temperature appeared consistently in Turkey as well as in Hard Federation. This relationship was shown also by the calculations based on the fresh weight, not reported in the tables.

The pH values of the juice of plants from corresponding day lengths were lower at the high than at the low temperature, excepting those of Turkey from the alkaline soil (groups 3 and 4 of table II). In previous experiments (**27**), and in others not reported here, lower values were consistently obtained at the higher temperatures, the difference being especially marked in Turkey. The evidence has indicated that increasing the temperature above the optimum for wheat tends to increase the acidity of the leaf juice by an amount which varies with the susceptibility of the particular variety to high-temperature injury (**27**).

HEADING PLANTS.—In the older plants of Hard Federation (table V),

as in the young plants of this variety (table III), there were no definite effects of temperature on the carbohydrate content. In the case of Turkey (table IV), however, the low-temperature plants continued to have the higher carbohydrate percentages, as they had had in the tillering stage (table II). The high-temperature plants of this variety (groups 2 and 4 of table IV), which failed to head and were cut while still in a leafy tillering stage after most of the others had been harvested, were generally characterized by relatively low concentrations of sucrose and relatively high concentrations of glucose.

Neither the nitrogen nor the pH values showed the differences due to temperature which had been so apparent in the young plants. The general absence of positive environmental effects on the chemical constituents of the older plants no doubt was due partly to the procedure of cutting the plants at different times as each lot reached the heading stage. It seems probable (**12, 13, 47, 48, 49**) that, at temperatures below 20° C., where wheat produces the best growth, the amounts of carbohydrates and nitrogenous compounds in the tissues are generally highest, but that exceptions to the generalization will be found as long as there are uncontrolled factors in the experiment.

Discussion

The problem in the foreground throughout these investigations, and the one which initiated them, was the determination of the chemical organization responsible for, or associated with, a particular type of growth. The various conditions of day length and temperature produced widely different types of plants, varying from the small, rapidly maturing plants of the spring variety at the long day to large, slowly maturing, vegetative types at the short day; from vigorous plants with a normal yield of grain at the natural day and low temperature to vegetative plants with culm elongation completely inhibited, such as those of the winter variety at the high temperature.

The data in tables II and III show that the leaves of the young, rapidly developing plants of the long day, which subsequently flowered at an abnormally early age, had the highest carbohydrate and lowest nitrogen percentages, and that those of the highly vegetative plants of the short day had the highest nitrogen and the lowest carbohydrate percentages. The accelerated heading at the long day was associated with very low yield of grain in Hard Federation, although with excellent yield in Turkey; and the vegetative condition at the short day was associated with fair yield in Hard Federation and with sterility in Turkey (table I). Thus there was no relation between the carbohydrate or nitrogen percentages and the subsequent yields of grain.

In the older plants of tables IV and V the carbohydrate and nitrogen percentages of the leaves bore no consistent relation to the type of growth. The leaves of the rapidly maturing long-day plants of Hard Federation (table V), which gave very low yields of grain (table I), had somewhat higher nitrogen values than the other plants of their respective groups; but the differences do not seem large enough to offer a satisfactory explanation of the extreme differences in growth habit and grain yield, and in fact are more probably due to the younger age at which the plants headed and were sampled. Nor were there any distinctive relationships characterizing the leaves of the vegetative short-day plants of Turkey whose heads failed to flower.

The leaves of the high-temperature plants of Turkey (groups 2 and 4 of table IV), most of which failed to produce heads and which were sampled at a late date while still in an abnormal leafy condition from which the plants never emerged, had total carbohydrate and nitrogen percentages of the same order of magnitude as were found in the leaves of the normal heading plants of the other groups. However, all of these sterile plants except those of the short day were characterized by unusually high glucose and low sucrose values. In the heading plants of both varieties the percentages of sucrose in the leaves were considerably higher than those of glucose; but in these vegetative, non-heading plants there was more glucose than sucrose in the leaves of all except the short-day plants. It is not believed that this relation has any general significance in connection with the tendency of the plant to produce grain, because there was no correlation of the glucose/sucrose ratio with fruitfulness in the other groups where poor yields were due to unfavorable light periods instead of to high temperatures.

Thus no consistent correlations were found between the grain production of the plants and the analyses of the leaves. However, the culm analyses of Hard Federation showed that the highest glucose concentration occurred consistently in those plants grown with the natural day where grain yield was highest (table V). The nitrogen percentages were generally somewhat lower in these culms than in those of the less fruitful plants of the short and long days. Unfortunately for a generalization, however, the culms of the heading plants of Turkey (groups 1 and 3 of table IV) did not show a correlation of glucose with fruitfulness, although the nitrogen percentages were again lower in the fertile culms of the natural- and long-day plants than in the sterile ones of the short-day plants.

NIGHTINGALE (39) found that the growth responses of tomato, salvia, and several other horticultural plants to different day lengths were correlated with the relative rather than with the absolute amounts of carbohydrates and nitrate-free nitrogen they contained. Other investigators

(**10, 38, 45, 46**) have expressed the opinion that the carbohydrate/nitrogen ratio would be found to explain the day-length responses of the plants with which they were working. From the chemical analyses of wheat, barley, oats, millet, and other plants made by ARTHUR, GUTHRIE, and NEWELL (**1**) and by BORODIN (**5**) however, it would seem that the effects of day length are not brought about by its effect on the carbohydrate/nitrogen ratio. Other evidence of limitations of the theory in explaining particular growth responses in terms of specific carbohydrate/nitrogen ratios has accumulated (**2, 19, 20, 40, 50**), suggesting that under many circumstances the proportionate amounts of these two classes of compounds reveal the physiological balance of the plant in only a general way.

In the present investigation the relatively low carbohydrate-nitrogen ratios of the leaves of the young plants which were subsequently more fruitful than those with higher ratios (table III), the normally high ratios found in the leaves of the vegetative high-temperature plants whose heading was largely inhibited (groups 2 and 4 of table IV), and the generally negative results with the leaves of the older plants, are not in accordance with the correlations which have sometimes been found between this ratio and fruitfulness in other plants. It may be significant that in the leaves of the young plants of both varieties there was always within each group a direct correlation of the magnitude of the ratio with rate of development; *i.e.*, the highest ratios in both varieties characterized the long-day plants, whose flowering was abnormally accelerated and whose vegetative development was inadequate, and the lowest ratios the highly vegetative short-day plants whose development was retarded; but it should be pointed out that since the plants of each group were sampled on the same day the long-day plants were in a more advanced stage of development. Only in the culms was there a consistent relationship between the magnitude of the ratio and the amount of grain produced by the plant (*cf.* table I). In Hard Federation, the culms of the long-day plants which produced the least grain had, without exception, the lowest ratios of each group, and those of the natural-day plants with the highest grain production had the highest ratios. In Turkey, both the natural- and the long-day plants at the low temperature gave good yields of grain, and the carbohydrate/nitrogen ratios of their culms were much higher than were those of the short-day plants which were sterile.

Summary

1. The leaves of young wheat plants, sampled at the same age in the tillering stage, had the highest carbohydrate and lowest nitrogen percentages at the long day, which accelerated culm elongation and flowering in both varieties investigated (Hard Federation, a spring wheat, and Turkey,

the winter variety). The lowest carbohydrate and highest nitrogen percentages were found at the short day, which retarded heading in both varieties and resulted in large vegetative plants, with reduced yield of grain in Hard Federation and complete sterility in Turkey (tables II, III, and VI).

2. Supplementary light of relatively high intensity (100 foot-candles) resulted in higher percentages of acid-hydrolyzable material, and consequently of total carbohydrates, and lower percentages of nitrogen, than the weaker light (15 to 50 foot-candles). There were no effects of this difference in light intensity on the external appearance or rate of development of the plants (tables II and III).

3. The sugar content of the young plants of the limed plots with a soil reaction of pH 8.1 was generally much reduced, and the acid-hydrolyzable material somewhat increased, compared with that of plants of the neutral soil. The nitrogen percentages were generally highest in the plants whose sugar content was reduced. The difference in soil reaction produced no visible effects on the appearance or rate of development of the plants (tables II and III).

4. The young plants of Turkey contained much more sugar, largely sucrose, at the low temperature where growth was normal than at the high temperature where the plants failed to head. In the case of Hard Federation, which headed and yielded fairly well at both temperatures, there was no significant difference in any of the carbohydrate fractions due to temperature. In neither variety was there any difference, attributable to temperature, in the acid-hydrolyzable material (tables II and III).

5. The nitrogen percentages of the young plants were higher at the low than at the high temperature, except in groups 3 and 4 of table II, but these also showed the higher percentages at the lower temperature when calculated on the residual or fresh-weight basis.

6. pH values of the juice of the young plants were generally highest at the short day and at the low temperature and lowest at the long day and the high temperature. They were not affected appreciably by the differences in light intensity or soil reaction (tables II and III).

7. These various relations between chemical composition and environmental factors which appear in the data for the young plants were frequently not found in the older plants, which were sampled on different days as they reached the heading stage (tables IV and V).

8. The leaves of Turkey at the high temperature, where culm elongation was largely inhibited in this variety, had total carbohydrate and nitrogen values of the same order of magnitude as were found in the leaves of the normal heading plants of the low temperature. The only peculiarity noted

in the leaves of these non-heading plants was their unusually high glucose-sucrose ratios (table IV).

9. Such correlations as were found between carbohydrate and nitrogen values and fruitfulness occurred in the culm analyses only, and had no counterparts in the leaf analyses. The culms of the natural-day plants of Hard Federation, which at both temperatures produced relatively high yields of grain compared with those of the short and long days, were consistently characterized by the highest glucose contents and lowest nitrogen percentages, and the highest carbohydrate/nitrogen ratios. Both the natural- and long-day plants of Turkey at the low temperature gave high yields of grain, and their culms were likewise characterized by high carbohydrate/nitrogen ratios as compared with those of the sterile short-day plants (tables IV and V).

The writers are indebted to Mr. DANIEL READY for the nitrogen analyses, to Mr. E. A. SIEGLER for suggestions responsible for the diagram in figure 4, and to Dr. MARY E. REID for suggestions in connection with interpretation of the data.

DIVISION OF CEREAL CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

LITERATURE CITED

1. ARTHUR, J. M., GUTHRIE, J. D., and NEWELL, J. M. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930.
2. AUCHTER, E. C., and HARLEY, C. P. Effect of various lengths of day on development and chemical composition of some horticultural plants. Proc. Amer. Soc. Hort. Sci. **21 (1924)**: 199–214. 1925.
3. BARTON-WRIGHT, E. C. Recent advances in plant physiology. Blakiston's Son and Co., Philadelphia. 1930.
4. BAYLES, B. B., and MARTIN, J. F. Growth habit and yield in wheat as influenced by time of seeding. Jour. Agr. Res. **42**: 483–500. 1931.
5. BORODIN, I. The influence of nitrogenous and mineral nutrition on the time of heading in barley and millet under conditions of different day lengths. Bull. Appl. Bot. and Pl. Breeding **27**: 171–193. 1931. [In Russian; English summary, pp. 194–195.]
6. BROWNE, C. A. Handbook of sugar analysis. Wiley and Sons, New York. 1912.
7. BYSTRIKOV, TH. V. On the influence of the soil reaction on the frost resistance of winter rye and winter wheat. Bull. Appl. Bot. and

Pl. Breed. **25**: 129–151. 1931. [In Russian; English summary, p. 149.]

8. CHIBNALL, A. C. Diurnal variations in the total nitrogen content of foliage leaves. Ann. Bot. **37**: 511–518. 1923.
9. DAVIS, A. R. The variability of plants grown in water cultures. Soil Sci. **11**: 1–32. 1921.
10. DEATS, M. E. The effect on plants of the increase and decrease of the period of illumination over that of the normal day period. Amer. Jour. Bot. **12**: 384–392. 1925.
11. DICKSON, J. G. The value of certain nutritive elements in the development of the oat plant. Amer. Jour. Bot. **5**: 301–324. 1918.
12. ————. The relation of plant physiology and chemistry to the study of disease resistance in plants. Jour. Amer. Soc. Agron. **17**: 676–695. 1925.
13. ————, ECKERSON, SOPHIA H., and LINK, K. P. The nature of resistance to seedling blight of cereals. Proc. Nat. Acad. Sci. **9**: 434–439. 1923.
14. FISCHER, H. Über die Blütenbildung in ihrer Abhängigkeit vom Licht und über die blütenbildenden Substanzen. Flora **94**: 478–490. 1905.
15. ————. Zur Frage der Kohlensäure-Ernährung der Pflanzen. Gartenflora **65**: 232–237. 1916.
16. GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.
17. ————, and ————. Further studies in photoperiodism. The response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871–920. 1923.
18. ————, BACON, C. W., and ALLARD, H. A. Photoperiodism in relation to hydrogen-ion concentration of the cell sap and the carbohydrate content of the plant. Jour. Agr. Res. **27**: 119–156. 1924.
19. GILBERT, B. E. Interrelation of relative day length and temperature. Bot. Gaz. **81**: 1–24. 1926.
20. HARVEY, E. M., and MURNEEK, A. E. The relation of carbohydrates and nitrogen to the behavior of apple spurs. Oregon Agr. Exp. Sta. Bull. 176. 1921.
21. HICKS, PHYLLIS A. The carbon/nitrogen ratio in the wheat plant. New Phytol. **27**: 1–46. 1928.
22. ————. Distribution of carbon/nitrogen ratio in the various organs of the wheat plant at different periods of its life history. New Phytol. **27**: 108–116. 1928.

23. HOOKER, H. D. A survey of investigations by American horticulturists on carbohydrate-nitrogen relations. Jour. Pomol. and Hort. Sci. **5**: 34–42. 1925.
24. HURD, ANNIE M. Hydrogen-ion concentration and varietal resistance of wheat to stem rust and other diseases. Jour. Agr. Res. **23**: 373–386. 1923.
25. ———. The course of acidity changes during the growth period of wheat with special reference to stem-rust resistance. Jour. Agr. Res. **27**: 725–735. 1924.
26. HURD-KARRER, ANNIE M. Changes in the buffer system of the wheat plant during its development. Plant Physiol. **3**: 131–153. 1928.
27. ———. Relation of leaf acidity to vigor in wheat grown at different temperatures. Jour. Agr. Res. **39**: 341–350. 1929.
28. ———. The formative effect of day length on wheat seedlings. Jour. Maryland Acad. Sci. **1**: 115–126. 1930.
29. ———. Comparative responses of a spring and a winter wheat to day length and temperature. Jour. Agr. Res. **46**: 867–888. 1933.
30. KLEBS, G. Über Probleme der Entwickelung. Biol. Centralbl. **24**: 257–267; 289–305. 1904.
31. ———. Alterations in the development and forms of plants as a result of environment. Proc. Roy. Soc. London B. **82**: 547–558. 1910.
32. ———. Über die Blütenbildung von Sempervivum. Flora **111/112**: 128–151. 1918.
33. KNIGHT, R. C. The carbohydrate/nitrogen ratio. Sci. Progr. **19**: 34–42. 1924.
34. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
35. LOEW, O. Zur Theorie der blütenbildenden Stoffe. Flora **94**: 124–128. 1905.
36. MASKELL, E. J., and MASON, T. G. Studies on the transport of nitrogenous substances in the cotton plant. II. Ann. Bot. **43**: 615–652. 1929.
37. MASON, T. G., and MASKELL, E. J. Studies on the transport of carbohydrates in the cotton plant. I. Ann. Bot. **42**: 189–253. 1928.
38. MAXIMOV, N. A. Experimentelle Änderungen der Länge der Vegetationsperiode bei den Pflanzen. Biol. Zentralbl. **49**: 513–543. 1929. Also in Bull. Appl. Bot. and Pl. Breeding **20**: 169–209. 1929.
39. NIGHTINGALE, G. T. Light in relation to the growth and chemical com-

position of some horticultural plants. Proc. Amer. Soc. Hort. Sci. **19(1922)**: 18–29. 1923. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.

40. PLATENIUS, H. Carbohydrate and nitrogen metabolism in the celery plant as related to premature seeding. Cornell Univ. Agr. Exp. Sta. Mem. 140. 1932.
41. REID, MARY E. Effect of variations in the amounts of available carbon and nitrogen on the growth of wheat seedlings. Amer. Jour. Bot. **16**: 770–779. 1929.
42. ————. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon. Bot. Gaz. **87**: 81–118. 1929.
43. SACHS, J. Stoff und Form der Pflanzenorgane. Arb. Bot. Inst. Würzburg **2**: 452–488. 1882.
44. SHAFFER, P. A., and HARTMANN, A. F. The iodometric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk, and other solutions. Jour. Biol. Chem. **45**: 365–390. 1921.
45. TINCKER, M. A. H. The effect of length of day upon the growth and reproduction of some economic plants. Ann. Bot. **39**: 721–754. 1925.
46. ————. The effect of length of day upon the growth and chemical composition of the tissues of certain economic plants. Ann. Bot. **42**: 101–140. 1928.
47. TOTTINGHAM, W. E. Temperature effects in the metabolism of wheat. Plant Physiol. **1**: 307–336. 1926.
48. WALSTER, H. L. Formative effect of high and low temperatures upon growth of barley: a chemical correlation. Bot. Gaz. **69**: 97–126. 1920.
49. WAŠNIEWSKI, S. Der Einfluss der Temperatur, des Lichtes und der Ernährung mit Stickstoff und Mineralstoffen auf den Stoffwechsel in den Keimpflanzen des Weizens. Bull. Intern. Acad. Sci. Cracovie 1914 (B): 615–686. 1914.
50. WORK, P. Nitrate of soda in the nutrition of the tomato. Cornell Univ. Agr. Exp. Sta. Mem. 75. 1924.

PHYSIOLOGICAL ASPECTS OF THE EFFECT OF CONTINUOUS SOIL AERATION ON PLANT GROWTH[1]

W. F. LOEHWING

Introduction

The experiments here reported deal with the effects of continuous aeration of sand and soil cultures. They are the outgrowth of nutritional studies which suggested that the beneficial effect of certain fertilizers was ascribable to the production of larger root systems and increased absorption of nutrients. Since it was known that soil aeration would increase the bulk of root systems, experiments were undertaken to determine whether it was thereby possible to duplicate the results of certain fertilizer treatments and thus disclose that such nutrients acted indirectly by enlargement of the root system. The results of the aeration experiments are reported separately because they reveal significant effects of soil air on plant metabolism. Data from nutritional studies will appear elsewhere.

CANNON and CLEMENTS have reviewed the earlier literature on plant responses to soil aeration, and they have extended our knowledge of that subject by a comprehensive set of experiments of their own (**19, 24**). The existing data indicate that the growth of most roots depends upon free soil oxygen (**12, 39, 45**) although some roots can develop anaerobically (**16**). Anaerobic roots are characteristically devoid of root hairs (**16, 18, 41**) and consequently absorptive processes differ from those of typical roots (**26, 28**). Even those roots with low soil oxygen requirements, however, are readily injured by moderately high concentrations of soil carbon dioxide (**14, 15, 27, 37**). Relatively high oxygen tensions are needed to offset otherwise toxic carbon dioxide concentrations about roots (**17**).

Improper composition of soil air manifests itself in reduced, slow-growing root systems, inadequate absorption, short-lived, discolored foliage and delay or failure of reproductive processes (**1, 11, 19, 30, 34**). The symptomatic complex arising from impaired gas exchange of roots reflects a general reduction in rate and magnitude of normal absorptive and growth processes. The great bulk of existing evidence thus indicates that roots are sensitive to variations in soil air. It also suggests that experimental manipulation of soil atmosphere provides an effective means of studying the rôle of root systems and their effect on the metabolism of the plant as a whole. It must be noted, however, that the preponderance of existing data deals chiefly with minimal oxygen requirements, carbon dioxide tolerance, and a general description of gross anatomical changes induced as

[1] An investigation aided by a research grant from the National Chapter of the Society of Sigma Xi.

critical concentrations of both gases are approached. The general character of the results of earlier workers nevertheless suggests that root activity is influenced fully as much by soil air as by water and essential mineral nutrients. The data given here indicate some of the metabolic differences between aerated and unaerated plants.

Procedure

The experiment comprised a double series of pot cultures, one of which contained coarse quartz sand and the other an ordinary fertile field loam. Two-gallon, glazed earthenware jars were provided with a tubed $3'' \times 3'' \times 1'$ porous basswood block to distribute the air supply. Pots of the sand series were filled with 12 kg. of sand and each uniformly aerated with a continuous stream of air approximating 100 liters daily. The main air stream was scrubbed by bubbling through two 10-liter bottles partly filled with water. These bottles also served to regulate the pressure and to humidify the air, thus maintaining the soil moisture of aerated pots very nearly equal to that of controls. Use of dry air was found to cause injury largely through its evaporational effect even at rather low rates of aeration. Humidity and rate of flow were further regulated by interposing a smaller water bottle between each pot and the main air supply (**6**). Sand cultures were initially given 500 cc. of a neutralized Knop's nutrient solution which was gradually brought up to a total of 1500 cc. at the end of the first month. The soil solution varied between pH 7.4 and 7.0 during the course of the experiment.

All pots were maintained at approximately constant weight by watering as needed. A control series was similarly treated except that aeration was omitted. A second series employing 10 kg. of fertile loam was also aerated in the foregoing manner in order to determine the effect of difference in soil type. Dwarf sunflowers (Manus variety) were used in both sand and loam cultures as well as inoculated Ito San soy beans in loam.

Initial analyses were made four weeks after germination while the plants were still in the vegetative phase. The final analyses were made in seven to nine weeks when the plants were in flower but before fruits had appeared. Sand and soil were carefully washed out of the jars in order to harvest the entire plant with minimum injury to the root system. Plants were separated into tops and roots by cutting at the ground line. Roots were carefully freed of solid matter, rinsed in distilled water, blotted, and allowed to air-dry for 30 minutes. Fresh weights were recorded and tissues were then comminuted by hand or Nixtamal mill.

Material for carbohydrate and nitrogen analyses was preserved in 80 per cent. alcohol. Soluble carbohydrates were first hydrolyzed and the precipitated copper estimated as glucose according to official methods (**10**).

The insoluble residue was refluxed with hydrochloric acid and further analyzed as starch (10). Nitrogen of dry material and sap was determined by the Kjeldahl-Gunning method. Calcium was titrated as oxalate against permanganate. Magnesium and phosphorus were estimated colorimetrically as phosphates in a solution made up of ammonium molybdate, sodium sulphite, and hydroquinon. Potassium was precipitated as chloroplatinate and iron estimated colorimetrically in amyl alcohol as thiocyanate.

Tissue for sap analyses was placed in sealed vials, immediately frozen at $-60°$ C. in dry ice. Tissue was rapidly thawed prior to extraction. Sap was expressed hydraulically and filtered through a double cloth filter at 10,000 pounds' pressure. Conductivity, hydrion, buffer, and oxidation measurements were made at 25° C. and these determinations as well as the freezing-point depression were commenced immediately after extraction of the sap (35).

Sap samples were immediately cleared, acid-hydrolyzed, and analyzed for reducing components which are recorded as glucose. Oxidase activity was determined colorimetrically with an indophenol reagent freshly prepared for each determination as follows: 0.144 gm. alpha-naphthol was dissolved in 10 cc. of 95 per cent. alcohol; then 0.209 gm. p-phenylenediamine-hydrochloride was added and the whole made up to 250 cc. with water, and finally neutralized with anhydrous sodium carbonate. Exactly 1 cc. of sap was added to 50-cc. portions of the reagent and permitted to stand for one hour in a 400-cc. beaker. Controls were similarly prepared except that sap was omitted. After standing for one hour, 50 cc. of 95 per cent. alcohol were added to dissolve the indophenol; both control and test sample were compared colorimetrically with a color standard containing 0.353 gm. indophenol per liter. Test samples generally showed formation of larger amounts of indophenol, and this quantity minus that formed in the controls is expressed in the tables as grams of reagent oxidized per liter of sap per hour.

Hydrion and buffer capacity were measured potentiometrically with a calomel half-cell and hydrogen electrode. Two 4-cc. samples of fresh sap were used for the acid and the alkali buffer curve respectively and the pH recorded after successive additions of 0.5-cc. portions of N/40 acid and alkali respectively. Osmotic pressures were computed from depression of the freezing-point determined by the Beckman method (35).

Data on sunflowers

The analytical data for sunflowers in the sand and loam series (tables I, II) disclose a general increase in size and weight of roots as well as tops in the aerated cultures. Seedling development is accelerated by aeration and the precocity thus established is maintained well into the reproductive

TABLE I

ANALYSES OF SUNFLOWERS GROWN IN SAND

	Age 29 days				Age 50 days			
	Tops		Roots		Tops		Roots	
A	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
Fresh weight, gm.	4.15	3.03	3.30	1.97	8.90	7.10	7.50	5.23
Top/root ratio	2.09	2.51	.....	.....	2.98	3.41	.....	.....
Dry weight, per cent.	13.90	10.39	8.37	6.29	14.34	13.93	5.70	5.57
B	Percentage of dry weight							
	%	%	%	%	%	%	%	%
Total sugars	12.71	19.59	17.56	29.27	11.54	12.79	15.63	20.50
Starch	19.91	25.32	12.33	20.64	13.67	15.90	33.94	34.40
Nitrogen	1.48	2.75	1.13	1.68	1.94	2.00	1.10	1.13
Ash	7.81	9.19	31.77	21.82	7.71	7.36	12.02	19.26
Calcium	2.48	2.92	1.65	2.16	3.16	2.31	1.12	1.84
Potassium	0.76	0.67	2.07	3.80	0.54	0.46	0.87	1.14
Magnesium	0.36	0.25	0.45	1.27	0.47	0.80	0.73	0.87
Phosphorus	0.14	0.15	0.07	0.23	0.17	0.18	0.34	0.10
C	Actual weights of chemical constituents per plant							
Dry weight, gm.	0.58	0.31	0.28	0.12	1.28	0.99	0.43	0.29
Total sugars, gm.	0.07	0.06	0.05	0.04	0.15	0.13	0.07	0.06
Starch, gm.	0.12	0.08	0.03	0.03	0.18	0.16	0.15	0.10
Nitrogen, mg.	8.50	8.60	3.10	2.10	24.80	20.00	4.70	3.30
Ash, mg.	45.10	28.60	87.69	27.06	98.40	72.90	49.40	56.00
Calcium, mg.	14.00	9.00	4.56	2.68	40.30	23.90	3.25	5.35
Potassium, mg.	4.39	2.08	5.51	4.63	6.91	4.56	3.72	3.32
Magnesium, mg.	2.08	0.78	1.24	1.58	6.02	7.94	3.12	2.53
Phosphorus, mg.	0.81	0.47	0.19	0.29	2.18	1.79	1.46	0.03

TABLE I—(*Continued*)

	Age 50 days				Age 29 days			
	Tops		Roots		Tops		Roots	
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
D	Grams per liter of expressed sap							
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Dry weight	56.12	39.82	21.28	22.33	63.18	43.38	19.46	12.58
Ash	5.26	6.48	2.37	3.97	5.16	5.75	2.96	4.46
Total sugars	2.07	2.99	15.15	11.70	16.88	10.85	8.20	6.40
Nitrogen	0.30	0.53	0.09	0.18	0.20	0.10	0.35	0.66
Indophenol	0.07	0.05	0.04	0.03	0.20	0.13	0.06	0.05
E	Osmotic pressure* and specific conductivity of sap at 25° C.							
Osmotic pressure	8.23	8.84	5.45	6.17	8.95	9.31	4.36	4.35
Conductivity	0.0116	0.0119	0.0094	0.0092	0.0116	0.0116	0.0092	0.0086
F	pH showing buffering action of 4 cc. of sap against fortieth normal reagent							
HCl n/40 3.0 cc.	4.46	4.46	3.93	3.99	4.61	4.50	3.70	-
2.5 cc.	4.63	4.56	4.05	4.15	4.47	4.63	3.80	
2.0 cc.	4.81	4.71	4.19	4.31	4.96	4.77	4.00	3.80
1.5 cc.	5.09	4.92	4.34	4.36	5.24	4.98	4.19	4.02
1.0 cc.	5.51	5.22	4.53	4.66	5.66	5.26	4.40	4.27
0.5 cc.	6.17	5.62	4.84	4.95	6.25	5.71	4.77	4.65
pH of pure sap	7.09	6 71	5 81	5.27	7.06	6.45	5.74	5.54
KOH n/40 0.5 cc.	7.66	7 54	7.86	6.02	7.48	7.07	7.38	7.07
1.0 cc.	7.97	7.90	8.91	7.49	7.74	7.45	8.51	8.05
1.5 cc.	8.21	8.14	9.40	8 31	7.96	7.72	9.01	8.55
2.0 cc.	8.42	8.35	9.72	8.81	8.13	7.93	9.34	8.85
2.5 cc.	8.61	8.54	10.50	9.31	8.28	8.13	9.55	9.16
3.0 cc.	8.78	8.72	11.10	9.63	8.43	8.26	9.72	9.31

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.

TABLE II

ANALYSES OF SUNFLOWERS GROWN IN LOAM
PLANTS SIXTY DAYS OLD

	Tops		Roots					
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
A					Actual weight in grams			
Fresh weight, gm.	4.43	3.75	4.58	3.61	gm. 4.43	gm. 3.75	gm. 4.58	gm. 3.61
Top/root ratio	1.59	1.34						
Dry weight	12.01%	11.18%	7.35%	8.49%	0.53	0.42	0.34	0.31
B	Percentage dry weight				Actual weight in milligrams			
	%	%	%	%	mg.	mg.	mg.	mg.
Total sugars	7.82	4.96	11.25	11.09	41.60	20.78	37.91	33.93
Starch	15.69	11.73	17.74	16.11	83.47	49.15	59.78	49.30
Nitrogen	5.61	3.79	2.08	1.82	29.75	15.88	7.01	5.57
Ash	17.46	15.75	8.57	9.60	92.89	65.99	28.88	29.38
Calcium	2.92	2.72	1.13	2.14	15.53	11.40	3.81	6.55
Potassium	1.90	1.15	0.87	1.26	10.11	4.82	2.93	3.86
Magnesium	0.48	0.47	0.92	1.19	2.55	1.97	3.10	3.64
Phosphorus	0.34	0.32	0.12	0.13	1.81	1.34	0.40	0.40
C	Grams per liter of sap				pH showing buffering action of sap†			
	gm.	gm.	gm.	gm.				
Dry weight	47.84	45.68	25.33	33.10	4.93	4.86	4.87	4.81
Ash	12.68	11.20	5.38	7.04	5.09	5.00	4.93	4.92
Total sugars	6.90	5.16	5.48	11.03	5.30	5.17	5.01	5.01
Nitrogen	6.28	1.22	0.33	0.38	5.57	5.40	5.15	5.05
Indophenol	0.0506	0.0466	0.0714	0.0774	5.93	5.76	5.37	5.22
					6.34	6.22	5.57	5.55
D	Sap osmotic pressure and conductivity*							
					6.97	6.78	5.88	5.49
Osmotic pressure	14.64	14.76	5.32	6.05	7.54	7.35	6.45	7.02
Conductivity	0.0290	0.0279	0.0112	0.0110	7.91	7.77	7.21	7.43
					8.20	8.13	7.69	7.66
					8.43	8.38	8.03	7.83
					8.63	8.59	8.35	7.96
					8.79	8.78	8.59	8.17

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.
† Readings as in table I, F.

phase. Although the controls are not invariably lower in percentage dry weight of each constituent, they are generally lower in corresponding absolute weights. Thus in the sand cultures (table I) the percentage ash in unaerated tops and roots is higher but the total weight of ash per plant is twice as great in young vegetative aerated plants. Differences in total ash tend to diminish with increasing age but aerated plants retain an appreciable excess even during senescence. Aerated plants absorb, transport, and utilize mineral nutrients more rapidly and efficiently than the controls if judged on the basis of rate of dry weight increase.

In connection with the essential inorganic materials, the small stature of the control plants explains the initially higher percentage of calcium despite the smaller total amount per plant in the sand cultures. There is an obvious tendency of calcium to accumulate in the roots of the controls in the maturation phases, both in sand and loam cultures. The absorption and internal distribution of magnesium is striking, occurring earlier and reaching the tops more rapidly in aerated young plants. This is evidenced by the fact that young aerated tops are much higher than the controls in magnesium while at the same time the unaerated roots run higher than aerated roots in percentage and total magnesium, both in sand and soil cultures (tables I, II). The original lag in magnesium intake by unaerated controls in sand was more than compensated, however, by rapid absorption during the seventh and eighth weeks. At the end of this time the total magnesium content of the mature controls in sand exceeded that of the aerated plants. The fact that this rapid intake of magnesium occurs late in the life of the controls and that it is coincident with a definite lag in potassium absorption may explain why the development of the sand controls fails to overtake that of the corresponding aerated plants (C, table I). Although less pronounced, similar results were observed in loam cultures. Acceleration in rate of magnesium intake is thus concomitant with a reduction in rate of potassium absorption.

Thus the initial percentage of potash is high in both aerated and unaerated plants but falls with increasing maturity, owing in part to the reason just given and also to the proportionately greater increase in dry weight over potash intake. Although percentage of potash in roots fluctuates, the aerated roots in sand are uniformly higher in absolute amounts of this element. Percentages of phosphorus are rather low in all young plants (**39, 40**) but increase significantly after the fifth week, especially in roots. Aerated plants not only run uniformly higher in total phosphorus but inaugurate reproduction earlier and maintain it longer than the controls.

The organic metabolites, namely, organic nitrogen, total sugars, and hydrolyzable polysaccharides, are uniformly higher in percentages but not

in absolute amount in all unaerated sand plants. Aerated loam plants, however, were higher in both percentage and total amounts of these constituents. Although not given in the tables, supplemental analyses showed a tendency for crude fiber and total amino nitrogen to be greater in aerated plants, a condition suggestive of more rapid assimilation. Since high sugar content coupled with abundant nitrate nitrogen generally characterizes impaired protein metabolism, this was probably also the case in the above instance.

It will be noted, however, that the soluble constituents of the sap, as indicated under dry weight composition thereof, tend to be higher in the tops of older aerated plants in sand and loam. Judging from the data on freezing-point depression by expressed fluids, their mineral constituents are osmotically more effective than their organic solutes. The specific conductivity of the sap of aerated and control plants, however, does not vary as greatly as might be anticipated from the preceding data. Both the conductivity and the buffer capacity of the sap were difficult to correlate with the pH and the mineral content of the plants. Aerated sunflower tops in sand appear better buffered than controls against acid, but as they reach maturity these differences in acid buffering diminish greatly. Mature unaerated roots on the other hand possess a better buffer capacity against alkali than aerated cultures of similar age. The greater free acidity of sap from unaerated plants suggests a higher acid content than in aerated cultures (**21**).

Total nitrogen dissolved in expressed sap is generally low but shows a tendency to accumulate in unaerated roots of sunflowers grown in sand (D, table I). This fact again implies not only slower translocation of solutes but also impaired nitrogen metabolism in controls, especially in older plants. On the whole, the sap of the plants showing the best development was marked by a higher oxidation potential, shown by the greater amount of indophenol produced. On the other hand, the top-root ratio in terms of fresh weight of the best developed plants was consistently lower in aerated plants than for the poorer controls of the same series (A, tables I, II).

Data on soy beans

The soy bean loam cultures (table III) exhibit several points of contrast with sunflowers, owing to the difference in feeding habits of the two species. Aerated soy beans displayed more abundant root nodule formation, and these plants were larger. In contrast with sunflowers, calcium was more rapidly absorbed and translocated to the tops. Sunflowers and soy beans were comparable, however, in the accumulation of calcium in the roots of older unaerated plants.

TABLE III

ANALYSES OF SOY BEANS GROWN IN LOAM

	Age 27 days				Age 56 days			
	Tops		Roots		Tops		Roots	
A	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
Fresh weight, gm.	2.18	1.87	1.70	0.94	3.95	3.17	3.03	1.45
Top/root ratio	2.44	4.51			3.51	4.73		
Dry weight, per cent.	13.29	12.76	6.99	5.65	17.69	15.24	6.56	7.00
B	Percentage dry weight							
	%	%	%	%	%	%	%	%
Total sugars	4.00	2.87	6.73	4.04	3.00	2.61	10.91	3.91
Starch	15.63	20.08	20.35	26.31	13.67	12.79	20.93	14.71
Nitrogen	4.92	3.41	4.82	2.20	3.45	3.98	3.62	3.11
Ash	9.98	11.25	22.00	32.25	9.15	11.87	8.94	13.16
Calcium	3.19	2.18	6.82	5.84	1.87	2.16	1.16	2.60
Potassium	1.70	1.04	3.65	4.51	1.39	1.44	0.78	1.56
Magnesium	0.24	0.23	1.42	3.18	0.41	0.43	0.81	1.07
Phosphorus	0.51	0.74	1.69	1.00	0.64	0.54	0.35	0.85
C	Actual weights of chemical constituents per plant							
Dry weight, gm.	0.29	0.24	0.12	0.05	0.70	0.48	0.20	0.10
Total sugars, mg.	11.80	6.80	8.00	2.12	21.00	12.60	21.80	3.90
Starch, mg.	45.00	47.90	24.40	13.90	95.60	61.70	47.80	15.00
Nitrogen, mg.	14.20	8.10	5.20	1.10	23.15	19.20	7.24	3.18
Ash, mg.	28.90	26.70	26.18	17.13	64.00	57.00	1.79	13.42
Calcium, mg.	9.25	5.21	1.00	3.10	13.09	10.43	2.30	2.65
Potassium, mg.	4.93	2.48	4.32	2.39	9.72	6.95	1.55	1.59
Magnesium, mg.	0.69	0.56	1.70	1.68	2.89	2.08	1.61	1.09
Phosphorus, mg.	1.47	1.77	2.02	0.53	4.48	2.61	0.70	0.87

TABLE III—(*Continued*)

ANALYSES OF SOY BEANS GROWN IN LOAM

	Age 27 days				Age 56 days			
	Tops		Roots		Tops		Roots	
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
D	Grams per liter of expressed sap							
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Dry weight	46.22	42.02	18.84	19.22	64.96	59.47	16.48	19.40
Ash	14.40	14.99	3.12	5.03	12.60	12.05	3.92	6.65
Total sugars	2.23	2.63	5.16	4.11	5.30	4.43	1.69	1.73
Nitrogen	1.21	3.45	0.24	0.39	2.73	2.00	0.68	0.68
Indophenol	0.04	0.03	0.04	0.02	0.08	0.10	0.06	0.04
E	Osmotic pressure* and specific conductivity of sap at 25° C.							
Osmotic pressure	.....	.....	.....	.....	15.12	9.44	4.96	5.69
Conductivity	0.0158	0.0159	0.0162	0.0164	0.0331	0.0314	0.0108	0.0138
F	pH showing buffering action of 4 cc. of sap against fortieth-normal reagent							
HCl n/40 3.0 cc.	.....	.....	.....	.....	7.06	6.48	9.03	8.04
2.5 cc.	.....	.....	.....	.....	5.07	5.08	3.21	4.16
2.0 cc.	.....	.....	.....	.....	5.18	5.20	3.45	4.37
1.5 cc.	.....	.....	.....	.....	5.31	5.32	3.74	4.64
1.0 cc.	.....	.....	.....	.....	5.45	5.45	4.06	5.00
0.5 cc.	.....	.....	.....	.....	5.61	5.58	4.46	5.45
					5.79	5.71	4.92	5.90
pH of pure sap	6.34	6.22	6.82	6.09				
KOH n/40 0.5 cc.	.....	.....	.....	.....	5.97	5.84	6.61	6.26
1.0 cc.	.....	.....	.....	.....	6.20	5.96	7.10	6.59
1.5 cc.	.....	.....	.....	.....	6.37	6.08	7.88	6.91
2.0 cc.	.....	.....	.....	.....	6.55	6.18	8.31	7.22
2.5 cc.	.....	.....	.....	.....	6.71	6.28	8.60	7.50
3.0 cc.	.....	.....	.....	.....	6.89	6.37	8.86	7.79

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.

Aerated and control soy bean seedlings did not differ greatly in magnesium content, nor was there any acceleration in its absorption by older control plants as observed in sunflowers. Aerated soy beans were higher in total magnesium. Aeration seemed to favor potassium intake throughout, while appreciable potash accumulation occurred in older control roots. Aerated young plants were characterized by rapid entry of phosphorus into roots but slow movement into the tops. The tops of older aerated plants were higher in phosphorus than the roots, however, thus showing a later acceleration in rate of translocation in aerated plants. Total phosphorus was higher in the entire aerated plants.

Although percentage composition of total sugars, starch, and nitrogen in soy bean loam cultures (table III) varied slightly from corresponding sunflower tests (table I, II), the absolute weights were analogous, showing a tendency of aerated plants to contain greater absolute amounts of these materials.

Specific conductivity of sap (E, table III) was variable, no definite tendency being observable. Sap osmotic pressures and pH were uniformly higher in aerated young and old tops. Tissue fluids of young aerated and unaerated soy beans in loam were all rather weakly buffered, but differences developed with increasing age. Unaerated tops of older soy beans were better buffered against alkali (F, table III). Aerated roots were poorly buffered against both acid and alkali, with a steep gradient in the median portion of buffer curves. Sap dry weight, ash, total sugars, and nitrogen ran appreciably higher in aerated tops but lower in aerated roots (D, table III). Top-root ratios were distinctly smaller for most aerated plants, owing chiefly to the greater bulk of the root systems.

Discussion

The preceding data not only coincide with earlier work as far as the general formative effects of soil aeration are concerned, but also suggest (in part at least) the physico-chemical explanation of the structural changes noted in earlier studies by other investigators. In this investigation, not only were roots found to be sensitive to changes in soil oxygen and carbon dioxide (**9, 29, 36, 43**), but continued soil aeration was also found to induce marked changes in the structure of tops and roots (**8, 11, 14, 31, 32, 41**), and to alter entirely the development of the root habit (**13, 16, 33, 46**). Abundant aeration favored increased branching and bulk of the root system (**3, 4, 25, 40, 44**) as well as profuse hair production (**11, 16, 18**).

Such marked differences in root systems were correlated with definite effect on the tops. Aerated tops were more vigorous in appearance, with larger, dark green leaves and higher yield (**2, 5, 7, 8, 32, 34, 42**). In contrast with studies of certain other investigators (**5, 43**) seedlings in aerated

sand cultures germinated and grew much more rapidly. This initial advantage over controls was maintained through maturation, as manifested by earlier and more prolonged flowering as well as by heavier fruiting.

Other investigators have shown that proper root aeration is especially important in relation to the reproductive phase of growth (**1, 22, 30**), abundant soil oxygen being known to favor the setting and development of fruit. On the other hand, oxygen deficiency and carbon dioxide toxicity in the soil are known to induce premature abscission of reproductive structures (**1**).

The data here reported indicate on the whole that the combined effect of increased oxygen tension and lowered carbon dioxide content of the soil initially alters mineral metabolism in a marked degree, which in turn subsequently alters the basic organic syntheses of plants. It is probable that most plants normally encounter a gradual reduction of oxygen and increase of carbon dioxide in the soil, and that the soil atmosphere is thus normally an important factor in the developmental cycle. If, as suggested by PARKER (**38, 39**), the normal increments in soil carbon dioxide during plant growth do not induce appreciable alterations in mineral metabolism, this is probably due to the maintenance of a liberal supply of soil oxygen. Certainly the data indicate that soil aeration alters the nutrition and organic syntheses of plants.

As the preceding analytical data on the effects of soil aeration are reviewed, it is apparent that aerated plants were characterized by a much more rapid absorption and transport of mineral nutrients. This in turn was followed by acceleration in the rate of nitrogen and carbohydrate accumulation, especially in loam cultures.

The initial absorption of magnesium and potassium in aerated cultures was especially rapid. Although the intake of calcium and phosphorus was slower, it continued longer and in total amount was well above that in the corresponding unaerated controls. These facts correlate well with the larger size of the finely branched root system and the profusion of root hairs of aerated cultures. It would be surprising indeed if this great increase in absorptive area of aerated roots did not result in enhanced feeding power. The greater indophenol formation by the tissue fluids of aerated tops suggests that these plants also metabolize their organic synthates more rapidly than the controls.

In addition to these compositional differences, aerated plants differed structurally from the controls. The striking effect of aeration on tops was acceleration of growth in early stages, attributable to increased length of basal internodes rather than to an increase in the number. Contrast in internodal distance diminished noticeably above the median nodes, however, and there was little or no difference in size of internodes near the

tips. Elongation continued in the controls after the aerated plants had started to flower. This response tended to reduce size differences when mature plants of both groups were compared. In fact, in the older soy bean plants in loam the percentage root dry weight of controls showed a tendency to exceed that of aerated plants. In 60-day old sunflowers continued dry weight increase of control roots also resulted in a lower top-root ratio, a response opposite to that found in the other instances. The physiological effect of aeration on tops thus appeared to be acceleration in development with earlier maturation rather than prolonged development.

With reference to the structural characteristics of aerated roots, it may be said that they were more fibrous, longer, and more highly branched. The root hair zone of aerated plants, although having more roots per unit surface, was less extensive longitudinally and the hairs were shorter lived, apparently because of the more rapid elongation of aerated roots. Internally the vascular tissues just above the root hair zone were not as definitely differentiated in aerated roots as in the controls. Other internal structural comparisons were difficult because of differences in size of aerated and control roots of similar age, but in general the walls of root cells in aerated plants were heavier, and such roots showed a slight tendency for intercellular spaces to increase as plants matured (7). Microchemical tests for starch and hemicelluloses were more pronounced in cells of aerated roots near the root hair zone.

Aeration of soil about the roots of aquatic angiosperms is known to produce formative responses similar to those described here. DEAN (25), working in this laboratory, has found that root systems of submerged aquatics, such as *Typha, Sagittaria,* and *Hibiscus,* increase greatly in size in aerated sand, clay, and muck. Roots in aerated soils were longer and more highly differentiated, as shown by heavy lignification of the new primary roots in many cases. In the main, unaerated roots were fibrous but less numerous than the corresponding fibrous elements of aerated root systems. Submerged (but not subterranean) water roots of *Typha* and *Sagittaria* were more numerous and profusely branched in unaerated soils. In every case larger tops were associated with more extensive root systems.

Perhaps the outstanding contrast between soil aeration of aquatic and terrestial plants is the effect of soil type. The structural characteristics of aquatics do not vary so greatly with soil differences as they do in land plants. Formative differences between aerated and unaerated sunflowers and soy beans grown in loam were never so marked as those in sand. Unaerated controls of soy bean, sunflower, and several other species not here discussed essentially overtook the aerated plants in size of tops in the maturation phase.

In conclusion it must be pointed out that the nature of the plant response

to soil aeration varies considerably from one species to another, and also varies with the degree of aeration. Sunflowers in pot cultures, although responding favorably to aeration rates less than 10 liters per kilogram of soil per day, were distinctly retarded in growth when this rate was doubled (**45**). Soy beans, were not so easily injured as sunflowers by rapid aeration, however, a contrast especially apparent in warm moist weather (**20**). In fact, tolerance of higher rates of aeration is increased as temperature rises.

It is interesting to note that similar responses have been recorded by other investigators for plants grown in solution cultures. Buckwheat, for example, is not benefited by rates of aeration known to be favorable to other plants (**23, 27**), and may in fact be injured if dry weight is taken as a criterion. Similar responses have recently been reported for aerated solution cultures of tomatoes. These also flowered and fruited later than the controls (**23**). The scanty data of other investigators on the reduced development of the root systems of plants injured by excessive aeration confirm those of the writer. Aeration uniformly increases the fibrous character of roots, but when over-aeration causes injury, the size of such injured roots is considerably diminished.

Summary

1. Aerated sunflower and soy bean plants grown in sand and loam differed from unaerated controls in the following ways:

(a) Taller in size and heavier in weight as a result of early rapid growth.

(b) Larger in root system, more fibrous, and more highly branched; root cells heavier walled and higher in reserve carbohydrates.

(c) More rapid nutrient absorption as shown by higher content of ash, calcium, potassium, and phosphorus per plant in terms of absolute weight of entire plants.

(d) Higher in total weight per plant of crude fiber, starch, total sugars, and nitrogen.

(e) More alkaline expressed sap in tops and roots; tops had a higher buffer capacity but the roots a lower buffer capacity against alkali and acid.

(f) Smaller top-root ratios in terms of fresh weight than in controls.

2. The difference between sand and loam cultures is expressed by a tendency of percentage composition of certain components to run higher in controls, even though absolute amounts of such constituents are consistently higher in aerated plants. Sap hydrion values were generally higher in plants grown on sand.

3. Moderate rates of continuous soil aeration with moist air increased size and growth rate of plants, but very rapid aeration had the opposite effect.

4. Species differed in their tolerance of soil aeration. The threshold of injury by rapid aeration is higher for soy beans than for sunflowers, especially when dry air is used.

UNIVERSITY OF IOWA
IOWA CITY, IOWA

LITERATURE CITED

1. ALBERT, W. B., and ARMSTRONG, O. Effects of high soil moisture and lack of soil aeration upon fruiting behavior of young cotton plants. Plant Physiol. **6**: 585–591. 1931.
2. ALLISON, R. V. The relation of aeration to development of the soy bean plant in artificial culture. New Jersey Agr. Exp. Sta. Ann. Rept. 338–344. 1921.
3. ———. Effect of aeration and continuous renewal of nutrient solutions upon the growth of barley and buckwheat in artificial culture. New Jersey Agr. Exp. Sta. Ann. Rept. 402–407. 1922.
4. ———, and SHIVE, J. W. Studies on the relation of aeration and continuous renewal of nutrient solution on the growth of soy beans in artificial culture. Amer. Jour. Bot. **10**: 554–567. 1923.
5. ———. The effect of aeration upon the development of barley in a heavy clay soil. Soil Sci. **17**: 97–105. 1924.
6. ANDREWS, F. M. Apparatus for aerating plants. Proc. Indiana Acad. Sci. **1919**: 165–167. 1920.
7. ———. The effect of aeration on plants. Proc. Indiana Acad. Sci. **1920**: 147–148. 1921.
8. ———, and BEAL, C. C. The effect of soaking in water and of aeration on the growth of *Zea mays*. Bull. Torr. Bot. Club **46**: 91–100. 1919.
9. ARKER, J. Die Beeinflussung des Wachsthums der Wurzeln durch das umgehende Mediums. Abst. Bot. Centralbl. **87**: 433–434. 1901.
10. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of analysis. 2d ed. Washington, D. C. 1925.
11. BERGMAN, H. F. The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps. Ann. Bot. **34**: 13–33. 1920.
12. CANNON, W. A. On the relation between the rate of root growth and the oxygen of the soil. Carnegie Inst. Washington Yearbook **15**: 74–75. 1916.
13. ———, and FREE, E. E. The ecological significance of soil aeration. Science n.s. **45**: 178–180. 1917.
14. ———. Root growth of *Prosopis velutina* and *Opuntia versi-*

color under conditions of a small oxygen supply. Carnegie Inst. Washington Yearbook **16**: 82–83. 1917.

15. ————. Reactions of roots of species with dissimilar habitats to different amounts of carbon dioxide in the soil. Carnegie Inst. Washington Yearbook **18**: 92–94. 1919.
16. ————, and FREE, E. E. Root adaptation to deficient soil aeration. Carnegie Inst. Washington Yearbook **19**: 62–63. 1920.
17. ————. Root growth in relation to the deficiency of oxygen or an excess of carbon dioxide in the soil. Carnegie Inst. Washington Yearbook **20**: 48–51. 1921.
18. ————. Experimental studies on roots. Carnegie Inst. Washington Yearbook **24**: 289–298. 1924, 1925.
19. ————. Physiological features of roots with especial reference to the relation of roots to the aeration of the soil. Carnegie Inst. Washington Publ. No. 368. 1925.
20. ————. On the upper critical concentration of oxygen in root growth. Science n. s. **61**: 118–120. 1925.
21. CERIGHELLI, R. Sur les échanges gazeux de la racine avec l' atmosphere. Compt. Rend. Acad. Sci. (Paris) **171**: 575–578. 1920.
22. CHESHUNT (England) Exp. & Res. Sta. Ann. Rept. **7**: 10–13. 1921.
23. CLARK, H. E., and SHIVE, J. W. Influence of continuous aeration upon growth of tomato plants in solution cultures. Soil Sci. **34**: 37–42. 1932.
24. CLEMENTS, F. E. Aeration and air content. The rôle of oxygen in root activity. Carnegie Inst. Washington Publ. No. 315. 1921.
25. DEAN, B. E. Effect of soil type and aeration upon root systems of certain aquatic plants. Plant Physiol. **8**: 203–222. 1933.
26. DEHERAIN P. P., and VESQUE, J. Recherches sur la respiration des racines. Ann. Sci. Nat. Bot. Serie 6, **3**: 327–343. 1876.
27. FREE, E. E. The effect of aeration on the growth of buckwheat in water cultures. Johns Hopkins Univ. Circ. n. s. **293**: 198–199. 1917.
28. ————, and LIVINGSTON, B. E. The effect of deficient soil oxygen on the roots of higher plants. Johns Hopkins Univ. Circ. n. s. **293**: 182–185. 1917.
29. HALL, A. D., BRENCHLEY, WINIFRED E., and UNDERWOOD, L. M. The soil solution and the mineral constituents of the soil. Phil. Trans. Roy. Soc. Lond. B **204**: 179–200. 1913.
30. HOLE, R. S., and SINGH, P. Oecology of Sal. Indian Forest Records **5**: 41–87. 1916.
31. HUNTER, C. The aerating system of *Vicia faba*. Ann. Bot. **29**: 627–634. 1915.

32. KNIGHT, R. C. The response of plants in soil and in water culture to aeration of the roots. Ann. Bot. **38**: 305–325. 1924.
33. LOEHWING, W. F. Effects of soil aeration on plant growth and root development. Proc. Iowa Acad. Sci. **38**: 71–72. 1931.
34. MCMURTREY, J. E. The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated solutions. Jour. Agr. Res. **38**: 371–380. 1929.
35. MORROW, C. A. Biochemical laboratory methods for students of the biological sciences. John Wiley & Sons, New York. 1927.
36. NOYES, H. A. The effect on plant growth of saturating a soil with carbon dioxide. Science **40**: 792. 1914.
37. ————, FROST, J. F., and YODER, L. Root variations induced by carbon dioxide gas additions to the soil. Bot. Gaz. **66**: 364–373. 1914.
38. PARKER, F. W. Carbon dioxide production of plant roots as a factor in the feeding power of plants. Soil Sci. **17**: 229–249. 1924.
39. ————. The carbon dioxide content of the soil air as a factor in the absorption of inorganic elements by plants. Soil Sci. **20**: 39–45. 1925.
40. SHIVE, J. W. Effect of aeration and continuous solution renewal on the salt requirements for soy beans in water culture. New Jersey Agr. Exp. Sta. Ann. Rept. 374–377. 1922.
41. SNOW, LAETITIA M. The effects of external agents on the production of root hairs. Bot. Gaz. **37**: 143–145. 1904.
42. STILES, W., and JØRGENSEN, I. Observations on the influence of aeration of the nutrient solution in water culture experiments with some remarks on the water culture methods. New Phytol. **16**: 182–197. 1917.
43. WACKER, J. Die Beeinflussung des Wachstums der Wurzeln durch das umgebende Medium. Jahrb. wiss. Bot. **32**: 71–116. 1898.
44. WEAVER, J. E., and HIMMEL, W. J. Relation of increased water content and decreased aeration to root development in hydrophytes. Plant Physiol. **5**: 69–92. 1930.
45. WIELER, A. Die Beeinflussung des Wachsens durch verminderte Partiärpressung des Sauerstoffs. Unters. Bot. Inst. Tübingen 1: p. 189. 1883.
46. WILSON, W. P. The production of aerating organs on the roots of swamp and other plants. Proc. Acad. Nat. Sci. Philadelphia 67–69. 1889. Abst. in Bot. Centralbl. **43**: 148. 1890.

POTENTIALLY UNLIMITED GROWTH OF EXCISED TOMATO ROOT TIPS IN A LIQUID MEDIUM

PHILIP R. WHITE

(WITH THREE FIGURES)

Introductory

In the cultivation of isolated tissues, whether plant or animal, it is necessary to distinguish between *growth* at the expense of the culture medium and *survival*, in which the medium plays only an inert or, at most, a secondary rôle. In the first case the nutrient is itself the limiting factor in determining the degree and to some extent the character of the increment obtained. Given a satisfactory nutrient, adequate replacement of exhausted nutrient, and adequate removal of excretory products, growth should continue indefinitely. In the second case increment may also take place for a time at the expense of materials reabsorbed from the older portions of the original explant, the medium acting essentially as an inert substratum only. As these reabsorbed materials are exhausted, the culture will gradually succumb, no matter how often the medium is renewed. An extended period of *growth* ordinarily implies the maintenance of a high level of metabolism. *Survival*, on the other hand, is ordinarily dependent on *minimal* metabolic rates, and is hence prolonged by depressant conditions such, for example, as moderate cold, low oxygen supply, etc.

The literature of the field of plant tissue cultures contains accounts of many experiments which clearly fall in the latter category. Reference need only be made to the work of HABERLANDT, BOBILIOFF-PREISSER, BÖRGER, CZECH, KUNKEL, PRÁT, ÚLEHLA, WHITE, *et al.* This literature has been reviewed more extensively elsewhere (**16**). All of these authors obtained *survival* of isolated tissues under various conditions, often for months, but no true growth. Perhaps the most striking example of this type of result is that of BAILEY and ZIRKLE (**1**) who, using sections of cambium from woody plants immersed in such non-nutrient media as paraffin oil, were able to maintain active protoplasmic cyclosis for many weeks. This is obviously a phenomenon of retarded metabolism only, a mere slowing down of the process of dying.

The experiments of KOTTE (**9**), ROBBINS (**12, 13**), ROBBINS and MANEVAL (**14, 15**), MALYSCHEV (**10**), and WHITE (**17–21**) are less easily classified. In many cultures of these workers active increment did take place, and in the work last cited the nature of the nutrient obviously did contribute quite largely in determining the extent and character of growth obtained. Only comparatively short culture periods were employed, however, and the absolute amounts of increment were small. It therefore is possible that this

increment may also have been dependent in part on materials carried over in the original fragment. The experiments of ROBBINS and MANEVAL (15), which represent the most extended periods (five months) and the greatest numbers of passages (ten) reported to date, strongly suggest that this was the case, since the observed mean growth rates decreased rather consistently from about 7.8 mm. per day in the first passage to 0.86 mm. per day in the tenth passage, after which the cultures were lost. Thus the growth rate at any passage bore an inverse relationship to the total amount of tissue formed up to that passage. This would appear to indicate that the rate of increment might have been determined by the concentration of some unknown material furnished to the culture from the tissues of the original fragment and gradually diluted in the multiplication of these tissues. If this be true, these cultures also were cases of *survival,* though for long periods. There are nowhere in the literature of plant tissue cultures accounts of any experiments which can with certainty be said to fall within the first category outlined above.

In view of this uncertainty and of the importance of the concept of *complete nutrients* in the use of a tissue culture method in the study of physiological and pathological problems, it has seemed desirable to determine whether or not a plant tissue can *grow* at the sole expense of a nutrient, without the *quantity* of material contributed by the original fragment entering as a limiting factor. A positive answer to this question will require that such a tissue be maintained in a state of active increase over a long enough period of time and through a sufficient number of passages to insure a dilution of all materials contained in the original fragment beyond the point where they could possibly be effective in determining growth rates. Such has been the purpose of the experiments presented in this paper.

Materials and methods

The present work represents essentially a repetition and extension of the earlier root tip cultures of ROBBINS (9, 10) and ROBBINS and MANEVAL (11, 12). Tomato was chosen as a test organism, since it was planned to use the tissue culture technique later in the study of certain diseases to which this plant is susceptible. It is assumed that wheat (18, 19) would have given similar results.

The methods of culture were approximately those outlined elsewhere (18, 19). The seeds to be used were removed under aseptic conditions from clean healthy fruit of the Bonny Best variety, grown under greenhouse conditions. They were usually germinated on sterile filter paper. As soon as the roots had attained a length of 5 mm., and some days before the cotyledons appeared, they were severed and placed in 125-ml. Erlenmeyer flasks containing 50 ml. of nutrient each. A single root was placed in each flask.

The nutrient medium used was approximately the same as that developed in previous work (19), with some slight modifications. It contained the following salts at the partial concentrations indicated:

SALT	MILLIMOLS
$Ca(NO_3)_2$	0.60
$MgSO_4$	0.30
KNO_3	0.80
KCl	0.87
KH_2PO_4	0.09
$Fe_2(SO_4)_3$	0.006

To this were added 2 per cent. by weight of sucrose, which for tomato was found experimentally to be superior to the dextrose used in previous experiments (18, 19), and the filtered extract of 0.01 per cent. by weight of dried brewers' yeast (18).

In making up the nutrient, the sequence of mixing the various ingredients appears to be important. The method found most satisfactory was to proceed as follows: The first four salts listed above, $Ca(NO_3)_2$, $MgSO_4$, KNO_3, and KCl were dissolved together in one flask. The KH_2PO_4 and $Fe_2(SO_4)_3$ were each dissolved separately. These were then mixed in a quantity of distilled water such as to make one half of the final volume of nutrient desired, and set aside until the yeast was ready, usually about two hours. The sucrose was dissolved in a quantity of water somewhat less than half the volume of the final nutrient and also set aside. The yeast was boiled for half an hour in 100 to 200 times its volume of distilled water, and filtered twice under suction, using no. 597 Schleicher and Schüll (or no. 1 Whatman) paper. The salts, sugar, and yeast extract were then mixed together, made up to volume, and distributed to the culture flasks, 50 ml. per flask. These were autoclaved immediately at 1 atm. pressure for 20 minutes. They were ready for use as soon as cool.

All cultures made previous to October 5, 1933, were placed on a bench in diffuse sunlight in a potting shed subject during the summer months to the temperature fluctuations of the outdoor atmosphere. On October 5th the cultures were transferred to a northeast laboratory where better temperature control was available. Here also they received diffuse sunlight during the daylight hours. The method of making the original cultures has been outlined above. Subcultures were made by cutting the roots into fragments each bearing one or more growing points. Each cutting was placed in a flask of fresh nutrient.

Experimental results

ROBBINS's experiments suggested the existence of some limiting factor present in the original fragment of tissue, diluted as this tissue multiplied,

not renewed under the cultural conditions employed, and hence fixing a limit to the amount of growth obtainable in such cultures. In examining further into the probability of the existence of such a factor and hence into the probable limits to which such excised root tips may be grown *in vitro*, data from two clones of cultures, designated as B and C, will be considered. Each clone was derived from a single root tip and was therefore genetically homogeneous.

In the preparation of clone B aseptic seeds of Bonny Best tomato were placed in a series of flasks. This series was started on November 2, 1932. Five days later the seed had begun to sprout and on November 11 the seedling roots were severed. The ages given in the following discussion are measured from this date. The apices were retained in the flasks, the seeds and seedlings being discarded. The fragments retained were about 10 mm. long. After some days the most vigorous examples were selected for further culturing. By November 21, that is 10 days after excision, the particular root under consideration had attained a length of 32 mm., with one branch. The primary root soon stopped growing, and the growing point turned brown, as often happened, especially in the earlier cultures. The lateral, however, continued to grow and to branch further. On November 30 the culture was transferred to a fresh nutrient but without cutting back.

A completely satisfactory experimental complex had not been developed at this time. Rather than risk the loss of the culture by transferring again to a nutrient of uncertain value, the flask was set aside while further experiments on methods were being made. The culture was left undisturbed for 145 days until April 24, 1933. At the end of this time the root had attained a total length, including branches, of 4564 mm., with 181 growing points. It was then removed and divided into 50 pieces, each peice being placed in a fresh flask. Twenty of the 50 subcultures were so made as to contain about 8 mm. of apical meristem each, without any mature tissue. These 20 grew much more satisfactorily than did the apices on larger masses of tissue,

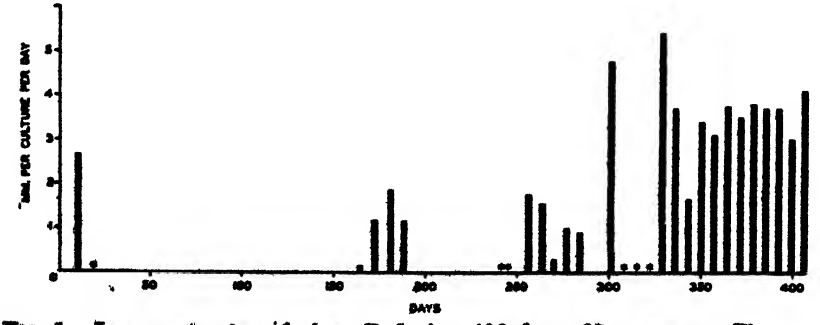


FIG. 1. Increment rates of clone B during 406 days, 29 passages. The asterisks represent passages in which no measurements were taken.

the old tissue apparently exerting a certain inhibitory action. All subsequent cultures were therefore made from these smaller pieces. The cultures grew quite consistently, though not very rapidly (fig. 1), remaining at the bottom of the flasks. On May 2, 11, and 18, subcultures were made, all but about 5 to 10 mm. of meristematic tissue being discarded at each transfer. They were then set aside again until July 10, an interval of 53 days. By this time they were brown, without active growing points, and apparently dead. Nevertheless, upon transfer to fresh nutrient, they resumed growth and put out fresh branches so that by July 14 it was possible to again sever the growing points, discarding all but about 5 mm. of each. Subcultures were made on July 25, August 1, 8, and 15. Up to this time the cultures had remained at the bottom of the flasks, growing only slowly, but branching with great profusion.

The low increment rate and high branching rate of those cultures which remain at the bottom of the flasks appear to be correlated with low respiration rate, but whether the fact of sinking to the bottom is itself the cause or an effect of some unknown condition remains to be determined. The two conditions are concomitant. Of the 50 cultures made on August 15, three floated to the top of the nutrient. These were all derived from no. 17 of the previous (12th) passage, and were designated as nos. 4, 5, and 6. Coincident with the floating of these individuals to the top of the nutrient, their increment rates quadrupled as compared with their previous values, the branching rate showing a corresponding fall. No. 4, for unknown reasons, ceased to grow after a week's time. No. 6 became contaminated with bacteria and had to be discarded. No. 5 remained in good condition and ultimately became the foundation for the entire clone. Although transfers on September 1 (14th passage) included material from 6 examples of the previous passage, all except the descendants of this one actively growing culture were eliminated by selection in the next two weekly passages. Detailed daily records were begun on September 28 in the 18th passage, when the culture was 322 days old, and were continued until it had reached the age of one year. In passages after that date the lengths of the roots at the beginning and end of each passage were recorded, giving a measure of the increment rates for each week, but without the daily detail. Records of the clone were discontinued at the end of the 28th passage, at an age of 405 days, but the clone is, at the time of preparation of this paper, alive and growing normally at an age of over 500 days.

Figure 1 shows the results of observations throughout 406 days of the life of this clone. Following the elimination of the slower growing members, the last 16 passages show a markedly *increased* mean growth rate over that of the previous 9 months. Figure 2 is of a single subculture photographed at the age of 367 days (passage, 12 days) and again 4 days later.

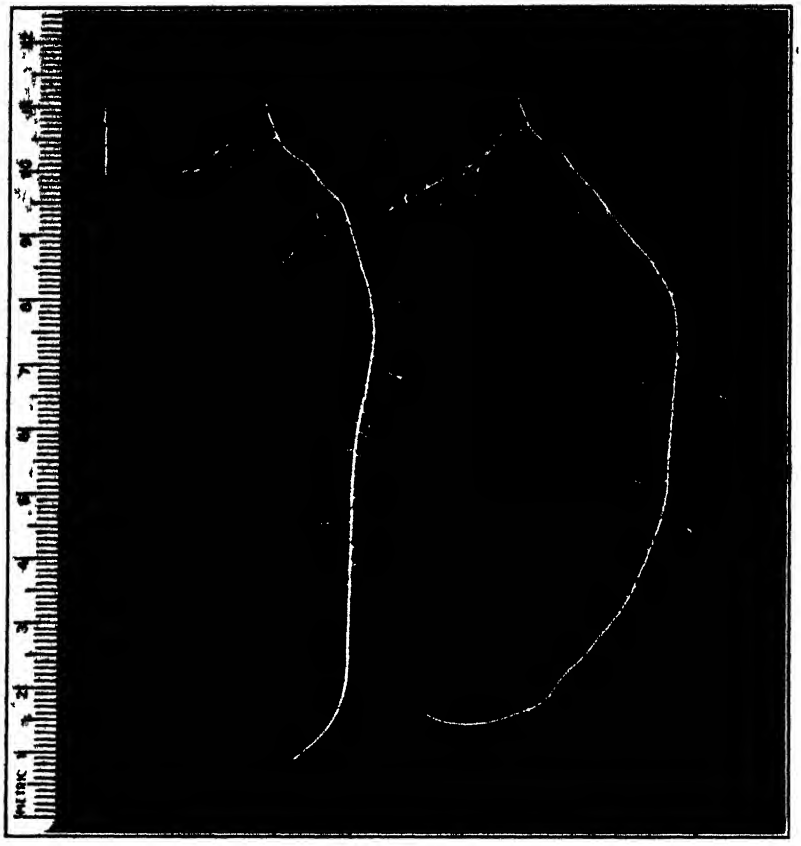


FIG. 2. A typical subculture of clone B, passage 23. The figure at the upper left shows the size of the fragment at the beginning of the passage, aged 355 days. At the center 12 days later, and at the right the same culture after 4 days more.

The increase both in length and in number of branches is evident, and represents what may be considered as approximately normal growth for tomato root tips. The fact that an isolated root tip can continue to maintain approximately normal activity at the end of a year's time in an artificial environment, is good presumptive evidence of its capacity to grow indefinitely under such conditions.

The records kept of clone C are somewhat more complete and continuous. Seeds of Bonny Best tomato were germinated aseptically on filter paper and on March 1, 1933, 25 severed root tips were placed in flasks of a nutrient (not the standard described above) which later proved only partially satisfactory. A single one grew, and this only poorly. On March 13 this one root was transferred to a more satisfactory nutrient in which it grew actively though somewhat unevenly. On March 29 it was divided into

16 subcultures and on April 21 two of these were selected and again divided into 42 and 24 pieces respectively. Neither the nutrient solution nor the culture method was at this time entirely satisfactory and experiments were conducted aiming at their improvement, using the cultures of this clone as experimental material. In the next 7 passages, therefore, the transfer periods and nutrients used were not uniform. With the 11th passage, at an age of 127 days, the nutrient solution and method of preparation outlined at the beginning of this paper were established as standard, and a period of one week chosen for the length of passage. The actual data must therefore be divided into two groups, the first embracing the first 10 passages, the second all passages subsequent to the 10th. The latter group should be much more nearly homogeneous and more reliable as a basis for drawing conclusions. Since at the date of preparation of this paper the clone is still being maintained, so that the data cannot be considered as a completed whole, a period covering 52 passages has been arbitrarily chosen for consideration in detail. The second homogeneous group of data, from the 11th to the 52d passage, thus embraces 294 days under standard conditions. These data are summarized in table I.

TABLE I

LINEAR INCREMENTS AND NUMBERS OF BRANCHES PRODUCED FROM A SINGLE ROOT DURING 52 PASSAGES

PASSAGES	TOTAL OBSERVED INCREMENT	TOTAL OBSERVED NUMBER OF BRANCHES	NUMBER OF PASSAGES	NUMBER OF DAYS RECORDED	MEAN PASSAGE LENGTH	MEAN BRANCHING RATE PER DAY	MEAN INCREMENT RATE PER DAY
	mm.						*mm.*
1–10	14,319	3,044	10	127(–52)*	13	0.385	1.91*
11–17	18,379	3,505	7	49	7	0.716	3.75
18–24	18,759	3,756	7	49	7	0.766	3.83
25–31	26,769	6,582	7	49	7	1.303	5.48
32–38	26,158	6,657	7	49	7	1.383	5.33
39–45	23,084	5,143	7	49	7	1.064	4.71
46–52	27,379	6,912	7	49	7	1.091	5.59
Total	154,847	35,399	52	421	.	0.958	4.37

* Detailed linear increment measurements were not made in the first 2 passages totaling 52 days. For that reason, although the first 10 passages totaled 127 days, the calculation of mean increment rate is based on 127–52, or 75 days only.

In 52 passages (421 days) the clone made a total measured increment, exclusive of all branches, of 154,847 mm. To this are to be added the increments of 35,399 branches 1 mm. or more in length. Since these average at least 5 mm. each, their total length would be about 175,000 mm. The total observed increment for 52 passages was, therefore, about 430 meters, or about 1300 feet. This was all derived from a single growing point approximately 10 mm. long. That a single root-tip can thus be maintained and made to multiply at approximately normal rates through 52 passages, is again strong presumptive evidence that it can be maintained indefinitely.

The details of the data further support this general presumption. In figure 3, A, the mean linear increment rates (exclusive of branches) are plotted as ordinates against the passage numbers as abscissas, while in figure 3, B, the mean numbers of branches formed per culture per passage are similarly shown. Each point on the curve of linear increments represents the mean value obtained from daily observations on 100 cultures over individual passages of 3 to 28 days. Since all passages after the 10th were uniformly 7 days in length, each point for the last 42 passages thus represents the mean of 700 observations. Each point on the curve of branching rates represents the mean number of branches on 100 cultures.

That portion of each curve representing the first ten experimental passages need not be considered in detail. The remainder, covering 42 passages under standard conditions, may be divided into five parts. Passages 10–14 show a steady rise both in increment rate and branching rate believed to have been brought about by gradual selection within the clone. Passages 14–17 are marked by an equally regular and very sharp fall in both indices. The cause of this was found to be lead poisoning from a faultily repaired water still. After this was corrected, passages 17–26 showed a quite regular increase, broken somewhat at the 19th and 20th passages, when transfers were made by one of the writer's colleagues,[1] and at the 24th and 25th passages when drastic culling, carried out in an effort to eliminate a troublesome brown discolored condition present in some cultures[2] resulted in discarding many cultures, thus lowering the average increments as estimated on the basis of the initial 100. Passages 26–39 then showed comparatively uniform growth indices, the mean linear increments oscillating quite regularly between extremes of 4.5 and 6.8 mm. per culture per day. The branching indices fluctuated somewhat more widely and erratically. A second

[1] It is a pleasure to acknowledge the help of Dr. L. O. KUNKEL who undertook the task of making the transfers, and of Dr. E. L. SPENCER who made the necessary measurements during this period.

[2] This condition was of such a nature as to suggest the presence of a bacterial infection. Attempts to find any organism, either by microscopic examination or by plating on various standard bacteriological culture media all failed, however. The incidence of this condition was greatly reduced by repeated selection, but its cause is still unknown.

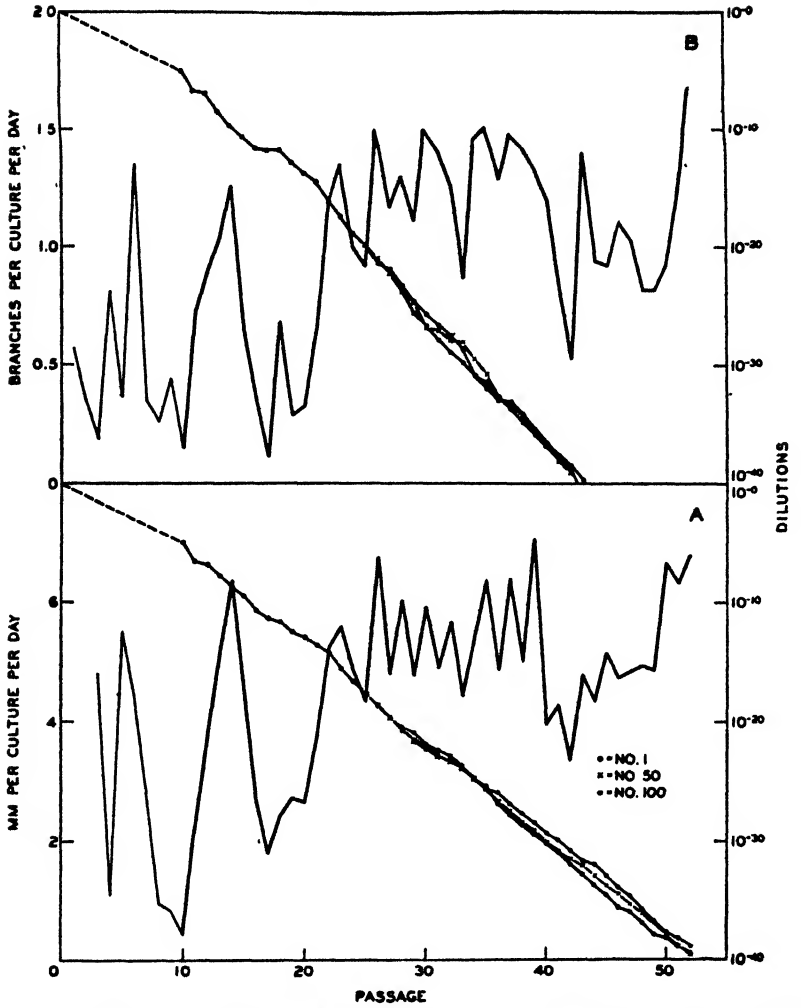


FIG. 3. A. Mean increment rates of clone C, in mm. per culture per day, plotted by passages. The corresponding dilutions of any material from the initial fragment distributed uniformly to all tissues formed are plotted for three individual cultures for comparison. B. Branching rates of clone C in numbers of branches formed per culture per day. The corresponding dilutions of any material from the initial fragment distributed uniformly to all growing points are plotted for the three examples as in A.

sharp drop in indices occurred in the 40th passage. The reason for this has not yet been satisfactorily traced, but the fact that both indices have subsequently recovered makes it clear that the drop was due to some uncontrolled external factor. The character of growth obtained at the end of the experiment was quite normal, the roots being clean, white, straight, with normal branching habit, and growing at rates which were not markedly sub-

normal. Individual cases of roots growing 30 to 40 mm. per day were fairly frequent. The factors which affect growth rates will be discussed more extensively elsewhere. The important fact brought out by these curves is that, unlike ROBBINS's cultures, the changes in growth rates can in no way be correlated with number of passages and can therefore *not* be interpreted as resulting from a time or dilution factor. This is perhaps even more clearly brought out by a comparison of clones B and C from the point of view of the relative effects of age and previous treatment. Clone B was approximately 200 days old in the 6th passage, while clone C had a similar age in the 21st passage. The linear indices at this age were for clone B 0.7, for clone C 3.7 mm. per culture per day. On the other hand, 134 days later the *simultaneous* indices were: clone B (18th passage) 5.3, clone C (23d passage) 5.6 mm. per culture per day. It is obvious from these figures that neither age nor member of passages, but simultaneity (and hence identity in environmental conditions) was the crucial factor in determining growth rates. It is the precise details of the environmental complex rather than any characteristics, other than genetic ones, inherent in the material of the explant, which determine the rates of growth at any moment.

Two strains of cultures have then been maintained for more than a year, one through 52 passages and the other through 30 passages. The measured tissue increment has been many thousands of times the original value. The mean growth rates have been clearly independent of origin, age, number of passages, previous treatment, etc., but closely dependent on the detailed variants in the artificial environmental complex. Hence it may be concluded with reasonable safety that the cultures have ceased to be dependent on any material factors carried over from the parent plant. The environmental complex used is therefore adequate for all requirements for growth of such tissues. The cultures have been shown to be capable of unlimited growth in such an environment.

The conclusion just drawn, that isolated root tips can be grown indefinitely under the experimental complex employed, may at first seem acceptable without further proof. Nevertheless, the data in the form presented above still leave a logical doubt as to its validity. The dilution of materials derived from the original explant, calculated as the ratio between initial tissue volume and total observed increment, has, even in clone C, been only of the order of $10/150{,}000 = 6 \times 10^{-5}$. It is well known that many materials: hormones, enzymes, viruses, etc., are effective at much greater dilutions than this. Even some of the essential nutrient elements, such as boron, become toxic above concentrations of the order of molar $\times 10^{-5}$. Copper, zinc, iodine, etc., are effective in even smaller quantities. It is hence quite conceivable that even at a dilution of 10^{-5} there may still remain sufficient quantities of limiting materials carried over from the original frag-

ment to be effective, although complete elimination of these materials would prevent further growth. In order to remove this doubt, it is therefore necessary, as stated in the introduction to this paper, to answer the question: "Can it be shown that the greatest possible crucial dilution for all conceivable materials of the original fragment must have been surpassed in the experiments under consideration?" This question has certainly not been answered in the foregoing section.

The experimental data do, however, furnish an affirmative answer if treated in a manner slightly different from that used above. If the pedigrees of three representative cultures of the last (52d) passage, nos. 1, 50, and 100, are traced back, it is found that all three were derived from culture no. 99 of passage 28, and further back from culture no. 57 of passage 11 when the standard environmental complex was introduced. Their histories from passage 11 to passage 28, through 17 passages, were thus identical. The detailed records show that this initial culture of the 11th passage, no. 57, was, when started, 7 mm. long, growing during the passage to a length of 36 mm. In passage 12 it was initially 11 mm. long, growing to 65 mm., the corresponding figures being 14 and 67 mm. for passage 13. The dilution of any material derived from this initial culture and uniformly distributed to all tissue formed therefrom during these three passages would thus be $7/36 \times 11/65 \times 14/67$, or 7×10^{-3}. The dilution obtained in this way for the 17 passages in which the history of the three examples was identical was about 3×10^{-12}, and the final dilutions for the three at the end of the 42 passages under standard conditions (52d passage) were 1.5×10^{-34}, 4.5×10^{-36}, and 5.3×10^{-36}. If, instead of considering the increment in volume of tissue, the numbers of growing points formed are taken as a measure of the dilution, the values obtained are still higher. During passages 11 to 15 the numbers of branches formed were 4, 8, 3, 22, and 19, giving a dilution of 2.5×10^{-5}. The final dilutions based on numbers of branches formed would then be 1.9×10^{-45}, 2.3×10^{-46}, and 1.6×10^{-45}. None of these figures include any of the dilution occurring during the first 10 experimental passages, which is roughly estimated to have been of the order of 10^{-5}. It is thus evident that whether this hypothetical limiting substance be considered as distributed to all tissues or only to growing points, its "dilution" at the end of the 52 experimental passages would be not less than approximately of the order of 10^{-40}.

Now, the pieces of tissue employed in starting each subculture weighed on the average about 2 mg. or 2×10^{-3} gm. fresh weight. A molecule of water has a mass of approximately 3×10^{-23} gm. If the root tips used as subcultures be considered to be made up of water only, such a piece would thus contain approximately $2 \times 10^{-3}/3 \times 10^{-23} = 0.67 \times 10^{20}$ molecules. If this be diluted as has been shown to have been the case in the cultures under

consideration to a value of the order of 10^{-40} times its original value, it is evident that there could still be only $10^{20} \times 10^{-40} = 10^{-20}$ part of one molecule of the material of the original root tip present in each culture of the 52d passage. This result obviously represents a *reductio ad absurdum*, and since the increment rate (fig. 3, A) at the end of the 52d passage has been equalled in only two previous passages, nos. 26 and 39, and the branching rate (fig. 3, B) is higher than at any previous time in the entire 400 odd days, the idea of any limiting factor in the form of a "hormone" or other material furnished to the culture from the parent plant is untenable. The results of the experiments of ROBBINS and MANEVAL, which seemed to indicate the existence of such a factor, must be explained in some other way.

This conclusion, of course, in no way affects the possibility of hormones being manufactured by the root itself. But if such exist they are not factors limiting the potential amount of growth obtainable in culture, and hence of no particular interest in the problem at hand. Moreover, so long as all the observed facts in a given series of experiments are, as in the present case, explainable without resorting to such hypotheses, and since the existence of such hormones can be demonstrated only by the behavior of cultures in their absence—a condition not demonstrably obtained in these cultures—the introduction of such hypotheses appears to the author to be an unnecessary encumbrance.

It appears, then, to have been demonstrated that the nutrient and the environmental complex employed in these experiments are adequate to sustain normal growth of such root tips indefinitely.

Discussion

It is important to note that in thus isolating a plant root from the parent plant and placing it in an artificial nutrient which is demonstrably adequate for its continued growth, a method is presented for an analysis of those factors by which the parent plant under natural conditions nourishes and controls this organ. It has been generally supposed, though without experimental demonstration, that the reciprocal relationship by which the aerial portions of the plant are supplied with salts and water by the roots and these in turn are supplied with the products of photosynthesis by the tops, was one necessary for the continued well being of both. The exact form in which these photosynthetic products were furnished to the roots has remained in doubt. It has also been doubtful just where the seat of synthesis of proteinaceous materials lay, whether in roots or aerial organs, and whether this process might not likewise be dependent on photosynthesis.

The present investigation has brought answers to some of these questions and furnishes a means not previously available of obtaining data pertinent to others. The interdependence of top and root is *not* a necessary one, at

least as far as the root is concerned, since these roots have shown unmistakably that they can be grown indefinitely without ever, even as seedlings, having been under the influence of tops. The artificial medium has provided adequate substitutes for whatever products the tops ordinarily send down to the roots. The photosynthetic product has been replaced by sucrose. The salts supplied have been those used for entire plants and, as was to be expected, their assimilation is quite independent of green tissues. The proteins required have evidently been manufactured in the roots, from the elements supplied in the nutrient. There remains, aside from possible traces of impurities, only one factor in the environmental complex supplied which is recognized as an unknown, namely, the extract of dried yeast. Whether this is itself replaceable by more easily analyzable materials remains to be seen.

It is interesting to compare the nutrient used in these experiments which has proved adequate to supply all the needs of a plant tissue, with the corresponding nutrients used for similar cultures of animal tissues. A great part of the work on the latter material has followed the methods introduced by HARRISON (8) and developed by CARREL. These methods will, therefore, be considered for comparison. In the work outlined above, the nutrient has consisted of three categories of materials: (1) the salt solution, (2) the carbohydrate, and (3) the organic material added as yeast extract. Of these, the exact constitution of the first two, representing 99.99 per cent. of the mass of the nutrient, is, aside from possible impurities, precisely known and can be varied at will. Only one component, the yeast extract, representing 0.01 per cent. of the entire mass, is of unknown constitution.

The nutrient generally used by CARREL (see CARREL, EBELING, FISCHER, ERDMAN, *et al.*), on the other hand, contains *no salt solution* and *no carbohydrate.* In its usual form it consists of two components, chicken serum making up from 50 to 70 per cent., and embryonic juice from 30 to 50 per cent., of the mass of the nutrient. A satisfactory modification used by EBELING (5) contained fibrinogen (prepared according to MELLANBY, 11) 12.5 per cent., chicken serum 37.5 per cent., and embryonic juice 50 per cent. Dry weights are not usually given for these constituents, so that they cannot be directly compared with the constituents of the plant culture nutrient. But it is clear that the animal nutrient differs radically from the plant nutrient in being made up entirely of complex organic materials. DREW (4) has replaced the serum of CARREL'S medium with a salt solution consisting of NaCl, $CaCl_2$, KCl, $NaHCO_3$, $MgHPO_4$, and $CaH_2(PO_4)_2$, and has replaced the embryonic juice with autolyzed tissue extract or tumor extract, with satisfactory results. In this case, the known elements are somewhat greater, but still represent less than half of the mass of the entire nutrient. It has also been shown that the embryonic juice can be replaced

by a fibrin digest prepared either by the action of pepsin (BAKER and CARREL, 2) or by controlled bacterial digestion (FISCHER and DEMUTH, 7). These represent, to a certain extent, attempts to reduce the medium to simple components which can be adequately analyzed. But even in the case of DREW'S salt solution it should be noted that, although the cations Na, Ca, K, and Mg are supplied, there has been no attempt to supply a balanced *anion* portion, the SO_4 and NO_3 ions being entirely omitted, and the PO_4 and CO_3 ions introduced only because of their buffer action. The other salt solutions occasionally used in such work: RINGER'S, TYRODE'S, etc., are, like DREW'S solution, made up of chlorides only, with PO_4 or CO_3 or both added only as buffers. Such unbalanced salt solutions, as has been experimentally demonstrated elsewhere (19), would be entirely inadequate for plant tissues.

It is, of course, difficult if not impossible to determine the exact constitution of such complex, essentially organic nutrients, while the determination of the constitution of the simple, essentially inorganic nutrient used in these plant cultures is comparatively simple. It is equally impossible to vary the concentrations of the active constituents, except in a very gross way. So long as such complexes as blood serum and embryo juice make up the greater part, or all, of the nutrient, the only nutrient variants available to the experimenter are varying proportions of these complexes. CARREL, BAKER, and others have made analyses which indicate that the proteoses play an important rôle in the activity of embryo juice, but these are again complex substances of undetermined constitution (FISCHER, 6). The complex nutrients have been retained because they fulfilled the two basic requirements of (1) complete adequacy for maintenance of unlimited growth, and (2) simplicity and duplicability of preparation. Perhaps if such a natural nutrient had been found in the early development of plant tissue cultures, it too would have been retained and used without further search. The latter field has, however, had a history quite the reverse of that of the animal field. Every attempt to find a natural culture medium comparable to that of CARREL, prepared from embryo juices, pressed saps, sieve tube exudates, endosperm fluids, digests, etc., has consistently failed with, in most cases, marked evidence of toxicity (see HABERLANDT, THIELMANN, ÚLEHLA, PRÁT, ROBBINS, MAYER, and others). Because of this failure, the workers in the plant field have been forced to adopt the reverse method, of starting with a simple medium of known constitution and, by a study of the individual constituents of this medium in their effects on growth and by the addition of new constituents, to build up an increasingly adequate, and at the same time precisely known, substratum and general complex for the maintenance of the cultures. The result has been a cultural complex which, as shown in the present paper, possesses both of the essential char-

acteristics of the animal complex, complete adequacy for the maintenance of unlimited growth, and simplicity and duplicability of preparation, and which in addition, as shown elsewhere (**18, 19**), possesses a flexibility far greater than that of the complex used in the older field.

Summary and conclusions

Tomato root tips, excised, and grown in a simple liquid medium of known constitution, have been maintained for over a year, and through 52 passages, in continuous active growth as measured by increase in number of growing points and total volume of tissue. One isolation has produced *in vitro* approximately 35,000 growing points and more than 400,000 mm. of tissue, from an initial fragment 10 mm. long. Calculations based on averages from approximately 40,000 separate observations on this clone give the theoretical dilution of any material of the original fragment as well beyond 10^{-40}, hence beyond the limits of molecular dilution. The entire material of most, if not all, of the ultimate cultures is therefore necessarily derived from the nutrient. This nutrient is thus demonstrated to be adequate for *all* growth requirements of such tissue. The growth rates have shown no consistent tendency to fall off, there being, on the contrary, a consistent increase in the early passages from 1.5 mm. per culture per day in the 1st to 6.8 mm. in the 26th passage, the corresponding value at the 52d passage being also 6.8 mm. per culture per day. It is thus proved that tomato root tips are capable of maintaining an independent, active, apparently normal existence *in vitro* for potentially unlimited periods of time, under such an artificial environmental complex as has been described here.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. BAILEY, I. W., and ZIRKLE, C. The cambium and its derivative tissues. VI. The effects of hydrogen ion concentration in vital staining. Jour. Gen. Physiol. **14**: 363–383. 1931.
2. BAKER, LILLIAN E., and CARREL, A. The effect of digests of pure proteins on cell proliferation. Jour. Exp. Med. **47**: 353–370. 1928.
3. CARREL, A., and BAKER, LILLIAN E. The chemical nature of substances required for cell multiplication. Jour. Exp. Med. **44**: 503–521. 1926.
4. DREW, A. H. A comparative study of normal and malignant tissues grown in artificial culture. Brit. Jour. Exp. Path. **3**: 20–27. 1922.

5. EBELING, A. H. Fibrin and serum as a culture medium. Jour. Exp. Med. 33: 641–646. 1921.
6. FISCHER, A. Gewebezüchtung. Handbuch der Gewebezellen *in vitro*. 3 Ausgabe. Müller and Steinicke. München. 1930.
7. FISCHER, A., and DEMUTH, F. Eiweissabbauprodukte als wachstumsfördernde Substanzen. Arch. exp. Zellf. **5**: 131–142. 1928.
8. HARRISON, R. G. Observations on the living developing nerve fiber. Proc. Soc. Exp. Biol. and Med. **4**: 140–143. 1907.
9. KOTTE, W. Kulturversuche mit isolierten Wurzelspitzen. Beitr. allg. Bot. **2**: 413–434. 1922.
10. MALYSCHEV, N. Das Wachstum des isolierten Wurzelmeristems auf sterilen Nährböden. Biol. Zentralbl. **52**: 257–265. 1932.
11. MELLANBY, J. The rate of formation of fibrin ferment from prothrombin by the action of thrombokinase and calcium chloride. Jour. Physiol. **51**: 396–403. 1917.
12. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
13. ———. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot. Gaz. **74**: 59–79. 1922.
14. ———, and MANEVAL, W. E. Further experiments on the growth of excised root tips under sterile conditions. Bot. Gaz. **76**: 274–287. 1923.
15. ———, and ———. Effect of light on growth of excised root tips under sterile conditions. Bot. Gaz. **78**: 424–432. 1924.
16. WHITE, P. R. Plant tissue cultures. The history and present status of the problem. Arch. exp. Zellf. **10**: 501–518. 1931.
17. ———. Plant tissue cultures. A preliminary report of results obtained in the culturing of certain plant meristems. Arch. exp. Zellf. **12**: 602–620. 1932.
18. ———. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid media. Plant Physiol. **7**: 613–628. 1932.
19. ———. Concentrations of inorganic ions as related to growth of excised root tips of wheat seedlings. Plant Physiol. **8**: 489–508. 1933.
20. ———. Plant tissue cultures. Results of preliminary experiments on the culturing of isolated stem-tips of *Stellaria media*. Protoplasma **19**: 97–116. 1933.
21. ———. Liquid media as substrata for the culturing of isolated root tips. Biol. Zentralbl. **53**: 359–364. 1933.

SALT CONCENTRATION AND REVERSIBILITY OF ICE-FORMATION AS RELATED TO THE HARDINESS OF WINTER WHEAT[1]

S. T. DEXTER[2]

(WITH SIX FIGURES)

Although the conditions of ice-formation in the tissues of plants have been the subject of numerous investigations, in the literature there appears to be no description of detailed quantitative experiments regarding the reversibility of the process of ice-formation. In several papers the reversibility or irreversibility of the process is implied. Thus, MÜLLER-THURGAU (**14**) showed that the freezing point of tissue was lower on the first freeze than on refreezing, which suggests irreversibility; NEWTON and GORTNER (**15**) specifically warned against refreezing the sample of expressed sap in determining "bound water." To the contrary, irreversibility might be implied by the technique used by STEINMETZ (**20**), who froze samples of alfalfa roots at an extremely low temperature, before expressing the sap to be used in "bound water" studies.

The question of reversibility of ice-formation in non-living colloidal systems has been repeatedly investigated. (*Cf.* JONES and GORTNER (**11**) for recent literature.) Such systems are sometimes indicated as "elastic gels" when reversible, and "non-elastic" gels when irreversible.

That the capacity for reversibility might well be significant in a consideration of winter-hardiness is brought out by casual observation of frozen plants as they thaw out. One may observe that, hardy and tender alike, the plants immediately after thawing have a water-soaked appearance; in the case of hardy plants, however, this water is reabsorbed in the course of a relatively short time and the plants regain their former appearance. If a temperature too cold for the survival of the hardy plants is used, this reabsorption does not occur.

When plant tissues freeze, the formation of ice removes water from the sap, thereby concentrating the solutes in the unfrozen water until the cryohydric point is reached. A study of the concentration of the minerals under such conditions is of interest, since "salting out" of colloids, often irreversibly, is a matter of common occurrence.

Experimental material

To examine this problem in an experimental way the following procedure was followed. Field plats of four varieties of winter wheat were seeded

[1] Contribution from the plant physiology laboratories, University Farm, University of Minnesota.

[2] National Research Council Fellow.

at the usual time and in the usual manner by the Agronomy Department of the University of Minnesota. No attempt was made to select varieties of peculiar varietal hardiness. However, the varieties in the probable order of their cold-resistance were as follows: (1) Minturki-Marquis hybrid (very hardy); (2) Minturki (very hardy); (3) Kanred (medium hardy); (4) Blackhull (medium hardy). These plats were not replicated and no pretense is made that, in their development, the typical hardiness of the varieties was necessarily developed. When samples were obtained at intervals throughout the autumn, every effort was made to obtain typical samples from the plats; however, on the last date (November 21) the ground was deeply frozen and covered with snow, and the samples were selected from small continuous areas which had been covered with paper after the ground froze but before the snow fell. The samples were prepared by removing the roots and leaf blades, together with any dried external leaf-sheaths. This material was discarded. The crowns were quickly washed by shaking with distilled water in a large beaker. In order to bring all of the samples to a comparable degree of dryness, the washed crowns were placed upon cellucotton pads and covered with a damp towel. In the course of an hour or two, the samples became almost free from surface moisture. During the process of weighing the samples, the crowns were further dried with cellucotton. After weighing, the samples were immediately wrapped in tin-foil. Percentage of dry matter was determined in similar samples by killing in an oven at 100° C. and drying *in vacuo* at 75° C.

Experimental methods

Determination of ice-formation

While several methods have been repeatedly used for the quantitative determination of ice-formation, it seemed that, for the experiment planned, the use of the calorimetric method would be most suitable. Recent work with this method is described in papers by Sayre (19), St. John (21), Meyer (13), and Robinson (17). Briefly, the method depends upon the heat of fusion of the ice formed; obviously, if, in a given sample, more ice is formed on one occasion than on the next, more heat will be required to melt the sample when more ice is formed. While several formulae have been devised, differing only slightly from one another, the following is used throughout this work.

$$\text{Ice, grams} = \frac{T_c\ (\text{T. C. cal.}) - T_s\ (\text{T. C. sample})}{80 - 0.5\ T_t}$$

where, T_c is the temperature change of the calorimeter, as indicated by the Beckman thermometer; T. C. cal. is the thermal capacity of the calorimeter, with 300 cc. of water; T_s is the temperature change of the sample, from the

frozen to the final melted condition; T. C. sample is the thermal capacity of the sample; and T_f is the temperature at which the sample was frozen, with the sign changed from negative to positive.

T. C. sample was calculated as follows: T. C. sample = grams water in sample + 0.35 (gm. dry matter) + 0.1 cal. The specific heat of the dry material in the sample was estimated at 0.35 calories per gm.; the 0.1 calorie represents the thermal capacity of the tin-foil used.

The calorimeter was a large silvered Dewar flask, heavily wrapped in cellucotton and stoppered with a thick cork stopper. Through the cork, a curved glass stirring rod and a Beckman thermometer projected into the flask. The top of the apparatus was further protected from air currents by a sheet of rubber through slits in which the stirrer and thermometer extended. 300 cc. of distilled water somewhat above room temperature were placed in the flask. To determine the thermal capacity of the calorimeter, stirring rod and thermometer, pieces of ice, frozen from distilled water, were dropped into the apparatus. Pieces with approximately the same heat of fusion as the samples were used, *i.e.*, about 15 gm. The average of 5 or 6 very closely agreeing replicates was used to compute the thermal capacity of the calorimeter.

Several methods have been suggested for the freezing of the samples and their transference to the calorimeter vessel. The following, which combines features of the methods of several investigators, was finally adopted. The samples of wheat crowns, weighing 20 gm., were wrapped in tin-foil and placed in tapered copper tubes. These tubes were placed in the freezing bath. If unwrapped samples were placed in tubes, it was found that considerable condensation of water vapor occurred on the inside of the tube. This would have introduced a very considerable error. Furthermore, the samples were found to freeze so firmly to the sides of the tube, if not wrapped, that is was impossible to remove them quantitatively or rapidly. When the large samples are wrapped in tin-foil, however, they melt with undue slowness, and the temperature equilibrium is markedly delayed. This delay is highly undesirable, for there is an inevitable, though slow, drift in the temperature of the calorimeter, due to the temperature difference between it and the air of the room. This drift may amount to as much as one one-hundredth of a degree a minute even when all precaution is taken to minimize it. To insure rapid melting in the calorimeter, the wrapped sample was removed from the copper tube after freezing thoroughly and the wrapper slit lengthwise, several times. The sample was handled with a paper pad kept in the cold room with the freezing bath, for that purpose. After slitting, the sample was replaced in the tube and freezing continued. With such treatment, thermal equilibrium was attained in the calorimeter in from 2 to 3 minutes, if the samples were vigorously stirred.

Large baths of alcohol-ice slush were prepared and kept in a cold room held at approximately the temperature desired. Alcohol or water was added to the baths to bring them to within one-tenth of a degree of the temperature required. The baths were readily kept at the desired temperature by frequent adjustments. All thermometers were calibrated in terms of one accurate thermometer. The Beckman thermometer was so adjusted that it read 21.4° C. below the actual temperature. It was read to the nearest hundredth of a degree.

Previous to freezing, the wrapped samples were stored at 2° C. overnight in the copper tubes. Duplicate samples of a single variety were placed in the freezing bath at the same time; after an interval of 30 minutes, a pair of duplicates of another variety was added and so forth. At the end of two hours of freezing, one sample was used for calorimetric measurements; the other, in its copper tube, was placed in a water bath at 2° C. where it remained for two hours. It was then refrozen for two hours at precisely the same temperature, when it was similarly used in the calorimeter. Thus, ice formation was determined in one sample after a single freezing treatment and in the duplicate after freezing, thawing and refreezing.

Freezing injury

After the sample and the calorimeter had reached thermal equilibrium, the sample with the 300 cc. of water was emptied into a beaker, the tin-foil removed, and the beaker, covered with a watch-glass, was transferred to a cold room held at 2° C. After 22 hours in this room, the electrical conductivity of the liquid was determined at 25° C. This gave an estimate of the freezing injury, according to the method of Dexter *et al.* (**5**, **6**).

Total extractable salts

The sample, with the liquid, was heated to boiling and promptly placed again in the cold room. At the end of 24 hours the electrical conductivity of the liquid was again measured, in order to determine the total extractable electrolytes in the sample. In several cases electrical conductivity measurements were made after longer intervals, up to six days; but the increase in extracted electrolytes was slight. Since relatively insoluble materials might go into solution on long standing, or since autolysis might occur, setting free electrolytes, the conductivity of the solution after 24 hours of extraction is considered to indicate the total amount of extractable salts in the samples.

Discussion of methods

From the preceding description, it is evident that the following values were determined for each of the four varieties of wheat on the various dates:

1. Grams dry matter in the sample.

2. Grams water in the sample, by difference.

3. Grams ice frozen on the first freezing treatment, and water unfrozen, by difference.

4. Grams ice frozen on the second (identical) freeze, after two hours thawing, and water unfrozen, by difference.

5. Electrolytes extracted in 22 hours after freezing injury, following single and double freezing treatment.

6. Total electrolytes extractable in 24 hours, following killing of the samples by boiling.

Determinations of the freezing points of the expressed saps were made on October 21 and November 22. The freezing points for the four varieties ranged from $-1.0°$ to $-1.25°$ on both dates, with considerable difference between duplicates. The sap of the hardier varieties appeared to have a slightly greater concentration of solutes as measured in this way.

Since the temperatures used in this experiment, $-7°$ C. and $-13°$ C., are above the cryohydric point of the salts commonly found in plants, it is likely that precipitation of the salts did not occur during freezing, and that the salts remained in solution in the unfrozen water. If this is the case, we are able to compute the average concentration of salts in the unfrozen water, *i.e.*, the concentration of salts to which the protoplasm is subjected on freezing. While it is rather commonly agreed that a certain amount of water is associated with the colloids in the plant, the opinion has been recently expressed in several papers that this water is probably partly if not wholly available for the solution of salts and sugars in the sap (BRIGGS (3), HILL (10), and GORTNER (8). Thus, while the addition of gum arabic, gelatin, or similar material to a sugar solution undoubtedly increases the freezing point depression, the water calculated as "bound" has not lost its colligative properties. Evidently if the colloid behaves in a manner similar to that of ordinary solutes, the amount of water bound by the colloid will depend upon the desiccating forces to which it is subjected, *i.e.*, less water will be "bound" at $-13°$ C. than at $-7°$ C. Furthermore, binding capacity is influenced by the nature of the solutes present. BRIGGS (2) found that the presence of sodium chloride and potassium chloride decreased the water binding power of gum arabic, and that calcium ions lowered the hydration capacity of gelatin more than did sodium ions.

It is not my purpose to discuss the status of the "bound" water problem. A full literature citation and review is given by GORTNER (8). No attempt has been made in these experiments to determine the bound water in the samples; an attempt has been made, rather, to determine the amount and reversibility of ice-formation at temperatures sufficiently low to differentiate by freezing injury, the hardiness condition of the plants.

TABLE I

ICE-FORMATION AND PERCENTAGE DRY MATTER ON VARIOUS DATES

VARIETY	−7° C. SERIES				−13° C. SERIES				
	FROZEN ONCE		FROZEN TWICE		FROZEN ONCE		FROZEN TWICE		
	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	DRY MATTER
	October 5								
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
Minturki-Marquis	12.00	5.52	14.19	3.33					12.4
Minturki	11.57	5.80	14.62	2.75					13.65
Kanred	13.25	4.39	15.66	1.98					11.8
Blackhull	12.48	5.18	15.00	2.66					11.7
	October 19 and 20								
Minturki-Marquis	8.97	7.67	11.81	4.81	14.27	2.37	14.79	1.85	16.8
Minturki	11.24	5.06	12.38	3.94	14.25	2.05	14.28	2.02	18.5
Kanred	11.33	5.31	13.00	3.64	14.63	2.01	15.19	1.45	16.8
Blackhull	12.32	4.46	13.54	3.24	15.27	1.51	15.25	1.53	16.1
	November 1 and 2								
Minturki-Marquis	9.50	6.42	11.39	4.53	13.31	2.61	13.49	2.43	20.4
Minturki	10.47	5.76	10.85	5.38	13.82	2.41	13.96	2.27	18.85
Kanred	10.99	5.46	12.17	4.28	14.25	2.20	14.95	1.50	17.75
Blackhull	11.31	5.17	12.11	4.37	14.48	2.00	15.00	1.58	17.6
	November 22 and 23								
Minturki-Marquis	9.91	5.94	11.32	4.52	13.27	2.58	13.46	2.39	20.75
Minturki	10.82	4.76	11.22	4.36	13.34	2.24	13.37	2.21	22.1
Kanred	10.84	5.18	12.03	4.00	13.92	2.10	13.96	2.06	19.9
Blackhull	10.87	5.24	11.40	4.71	13.96	2.15	14.20	1.91	19.45

Experimental results

Table I shows the percentage of dry matter, the amount of ice frozen, and of water unfrozen on first and second freezing treatment of each sample of the four varieties of winter wheat on several dates. Minturki-Marquis and Minturki are known to be hardier than Kanred and Blackhull. Table II summarizes table I. The following facts appear:

TABLE II

SUMMARY OF TABLE I

DATE	−7° C. SERIES			−13° C. SERIES		
	TOTAL UNFROZEN WATER			TOTAL UNFROZEN WATER		
	FROZEN ONCE	FROZEN TWICE	RATIO	FROZEN ONCE	FROZEN TWICE	RATIO
	gm.	*gm.*		*gm.*	*gm.*	
October 5	20.89	10.72	0.513			
October 19	22.50	15.65	0.695	7.94	6.85	0.853
November 1	22.81	18.56	0.814	9.22	7.78	0.845
November 22	21.12	17.59	0.833	9.07	8.57	0.945

DATE	TOTAL DRY MATTER	UNFROZEN WATER PER GRAM OF DRY MATTER			
		−7° ONCE	−7° TWICE	−13° ONCE	−13° TWICE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
October 5	9.81	2.13	1.09		
October 19	13.64	1.65	1.15	0.582	0.502
November 1	14.94	1.53	1.24	0.617	0.521
November 22	16.44	1.29	1.06	0.552	0.521

1. There is invariably more water frozen on the second freezing than on the first, but this tendency becomes less marked as the hardening process proceeds. Figure 1 shows this in graphic form. The amount of water left unfrozen on the second freezing treatment is 51 per cent. of that left unfrozen after one freezing treatment on October 5 (−7° C.) As the plants harden, the values approach each other more closely, until on November 22 the ratio is 83 per cent. This indicates that the ice-formation becomes more nearly reversible as the plants harden in the field, and that the period allowed for reabsorption of water after freezing is ineffectual in the case of the tender plants.

2. There is almost invariably more water left unfrozen in the hardy varieties than in the less hardy ones.

3. The amount of water left unfrozen per gram of dry matter is greater,

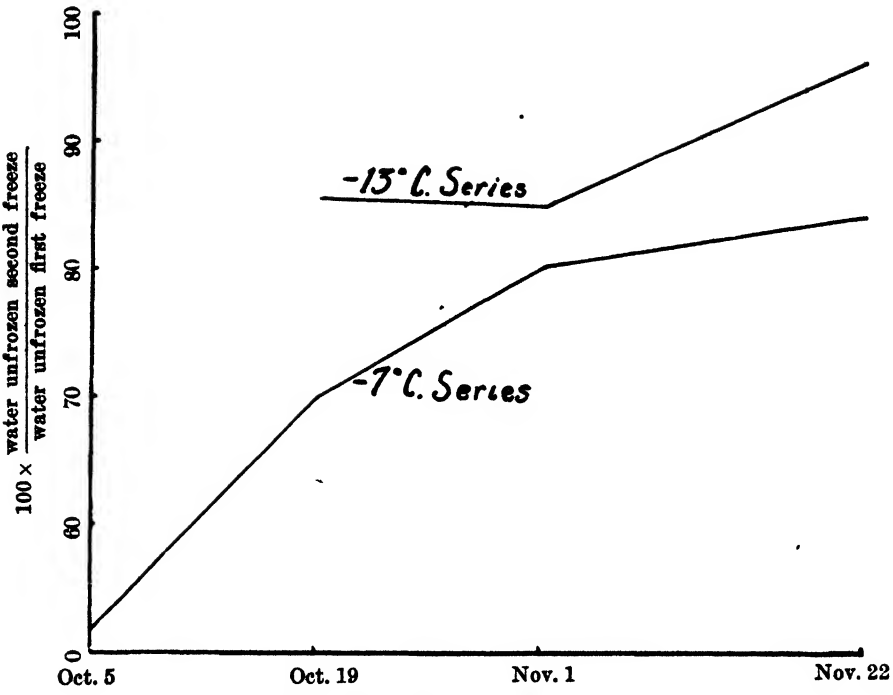


FIG. 1. Ratios of $\frac{\text{unfrozen water, second freeze}}{\text{unfrozen water, first freeze}}$ are shown on the vertical axis. This gives a measure of the reversibility of water withdrawal to form ice. On October 5 there was about twice as much water left unfrozen after the first freeze (−7° C.) as after the second. As the season advanced the amounts became more nearly equal.

on the first freeze, −7° C., when the plants are in the unhardened condition than when they are hardened (MEYER, 13). On the second freeze, −7° C., there is little difference in hydration between the hardened and unhardened condition. The same may be said of the −13° C. freezing treatments. There appears to be little evidence that there is a greater hydration of the colloids in the hardened state; but it would appear that the hydration is more stable. Some indication of a reason for this greater stability will be brought out later, when mineral concentrations are considered.

Table III shows the amount of water, dry matter, and extractable electrolytes in 20-gm. samples of wheat crowns of four varieties taken from the field on different dates. These samples, with the exception of those on the last date were never frozen in the field. The value for extractable salt is the specific conductivity ($K \times 10^5$, 25° C.) of the solution into which the electrolytes diffused. These conductivity values are average of duplicates, single and double freeze, −7° C. which rarely varied more than one per cent. from the mean. The last two columns of the table show the computed

TABLE III

TOTAL WATER, DRY MATTER, AND EXTRACTABLE ELECTROLYTES ($K \times 10^5$, 25° C.) IN SAMPLES ON VARIOUS DATES

DATE	VARIETY	WATER	DRY MATTER	EXTRACTABLE SALTS	SALT PER GRAM WATER	SALT PER GRAM DRY MATTER
		gm.	*gm.*	$K \times 10^5$	$K \times 10^5$	$K \times 10^5$
October 5	Minturki-Marquis	17.52	2.48	109.0	6.21	44.0
"	Minturki	17.27	2.73	116.0	6.67	42.5
"	Kanred	17.64	2.36	120.7	6.83	51.2
"	Blackhull	17.66	2.34	115.6	6.54	49.3
	Average			112.8	6.56	46.75
October 19	Minturki-Marquis	16.64	3.36	84.4	5.07	25.1
"	Minturki	16.30	3.70	90.2	5.52	24.4
"	Kanred	16.64	3.36	100.5	6.03	29.9
"	Blackhull	16.78	3.22	91.0	5.42	28.3
	Average			91.5	5.51	26.42
November 1	Minturki-Marquis	15.92	4.08	72.0	4.53	17.65
"	Minturki	16.23	3.77	79.5	4.89	21.10
"	Kanred	16.45	3.55	81.6	4.98	22.95
"	Blackhull	16.48	3.52	78.6	4.77	22.35
	Average			77.9	4.79	21.01
November 22	Minturki-Marquis	15.85	4.15	64.3	4.05	15.50
"	Minturki	15.58	4.42	70.1	4.51	15.85
"	Kanred	16.03	3.97	72.4	4.52	18.29
"	Blackhull	16.11	3.89	72.2	4.48	18.55
	Average			69.8	4.39	17.05

amount of soluble salt in each gram of water or of dry matter, respectively.

From the table, the following facts appear:

1. The water in the samples decreased as the season advanced and the dry matter increased. This has been the finding of workers, generally, in this field.

2. The total extractable electrolytes decreased regularly with each of the four varieties.

3. The salt per gram of water in the plant decreased as the season advanced, in spite of the fact that the amount of water decreased. That is, the concentration of salt in the sap, assuming all water to be free for solvent purposes, decreased regularly (figure 2).

4. The soluble salt per gram of dry matter decreased very markedly as hardening proceeded (figure 3). Furthermore, the amount of extractable salt per gram of dry matter appears to be related to the hardiness of the

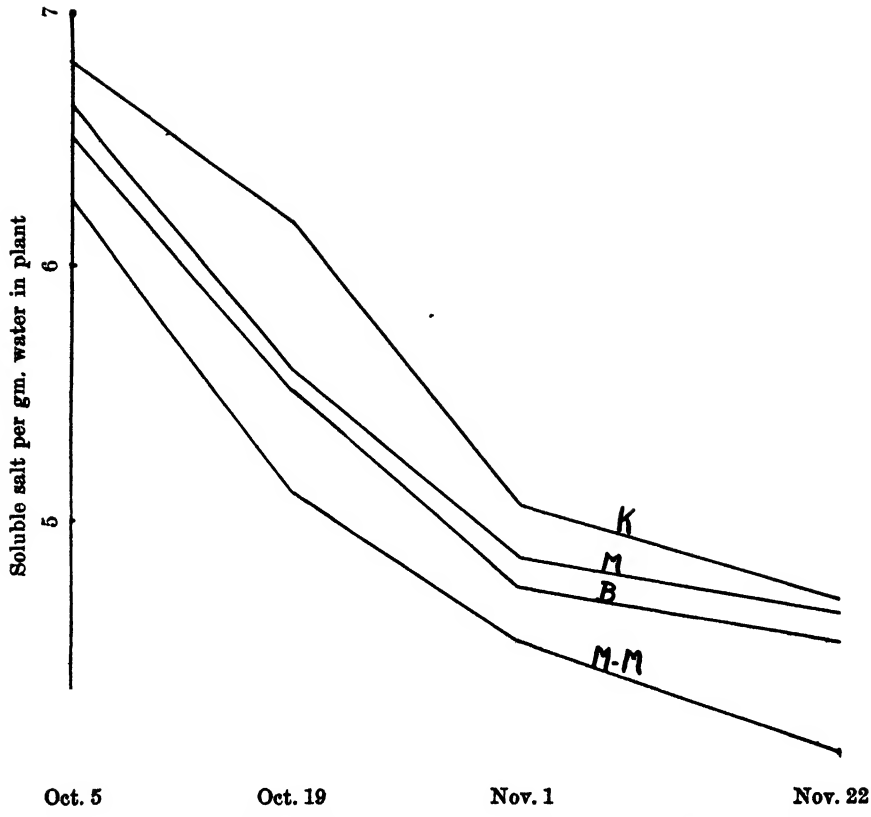


FIG. 2. Total extractable electrolytes from each sample (expressed as specific conductivity $\times 10^5$) was divided by the grams of water in that sample to give the values on the vertical axis. This gives the relative concentrations of salts in the cell sap on the dates shown. A progressive decrease in salt concentration is shown in all four varieties. M-M = Minturki-Marquis; M = Minturki; K = Kanred; B = Blackhull.

variety, and is generally lower in the hardy ones (ROSA (18), CHANDLER (4), GORKE (7)).

Since the amount of unfrozen water was determined in each sample together with the amount of soluble salt, one may readily calculate the concentration of salt per gram of unfrozen water. Furthermore, measurements were taken of the exosmosis of electrolytes following freezing, to give an estimate of freezing injury. Table IV presents these values. The "salt per gram of unfrozen water" was calculated by dividing "extractable salt" (table III) by "grams unfrozen water" (second freeze) (table I). The freezing-exosmosis conductivity values are the average of the same duplicates, except for the values given for October 5, where the value obtained for the sample frozen twice was used. On this date, the sample frozen twice was

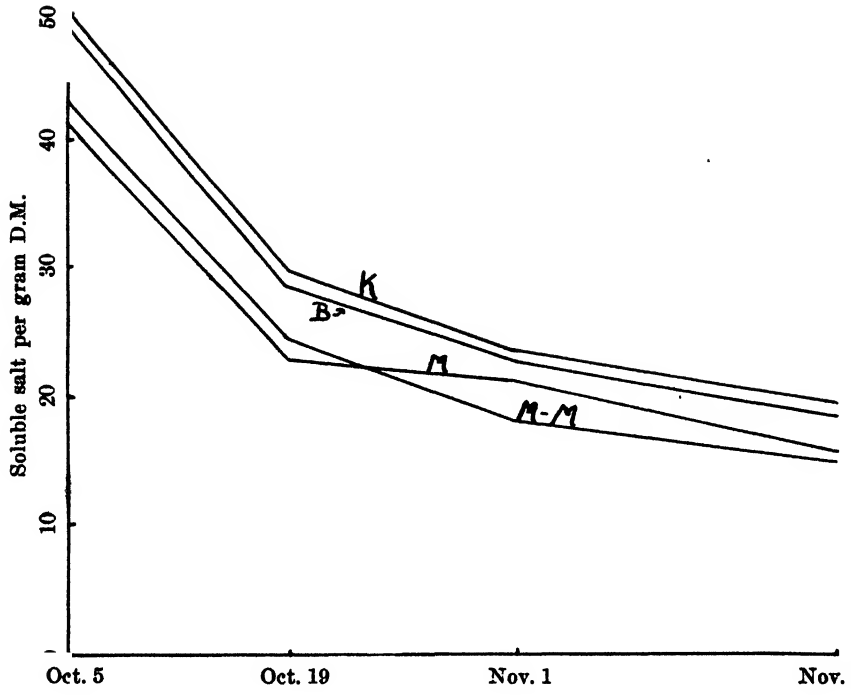


FIG. 3. Progressive change in ratio of $\frac{\text{soluble electrolytes}}{\text{grams dry matter}}$ in the four varieties of winter wheat. M-M(Minturki-Marquis hybrid) and M(Minturki) are hardier than K(Kanred) and B(Blackhull). The hardier varieties have in general a lower ratio of soluble salts to dry matter. In all varieties the ratio decreases greatly as the season advances and the plants increase in hardiness.

materially more injured than the sample frozen once only, and thus the conductivity value used represents the true injury more closely than would the average.

From table IV the following facts appear:

1. In a given variety, as hardening proceeded, the concentration of salts in the unfrozen water decreased to about one-third the value found in the early fall (−7° C. freeze) (figure 4).

2. The concentration of salts in the unfrozen water is regularly greater in the varieties known to be hardier by agronomic experience. Almost invariably the freezing-exosmosis values for frost injury are in the same order as the values for salt concentration in the unfrozen water. Figure 5 shows this in a graphic way.

From these results, it would appear that freezing injury may well be closely correlated with the concentration of salts in the unfrozen water. This theory is, in a way, by no means new. GORKE (7), HARVEY (9), and

TABLE IV

CONCENTRATION OF SALTS PER GRAM OF WATER LEFT UNFROZEN, TOGETHER WITH FREEZING-EXOSMOSIS VALUES OF INJURY FROM FREEZING

VARIETY	OCTOBER 5		OCTOBER 19		NOVEMBER 1		NOVEMBER 22	
	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY
				−7° C. series				
	gm.	$K \times 10^5$	*gm.*	$K \times 10^5$	*gm.*	$K \times 10^5$	*gm.*	$K \times 10^5$
Minturki-Marquis	32.7	67.7	17.5	29.6	15.88	23.3	14.37	17.9
Minturki	42.2	72.5	23.0	42.6	16.25	31.7	16.02	20.1
Kanred	60.9	91.8	27.8	48.0	19.02	33.9	18.10	21.65
Blackhull	43.4	84.3	28.0	48.6	17.88	31.0	15.36	22.9
				−13° C. series				
Minturki-Marquis			47.7	63.0	29.7	47.2	29.3	42.2
Minturki			46.0	74.1	37.2	55.9	33.2	43.2
Kanred			71.7	85.2	58.6	63.2	37.3	46.9
Blackhull			61.3	75.5	51.2	57.8	42.0	48.2

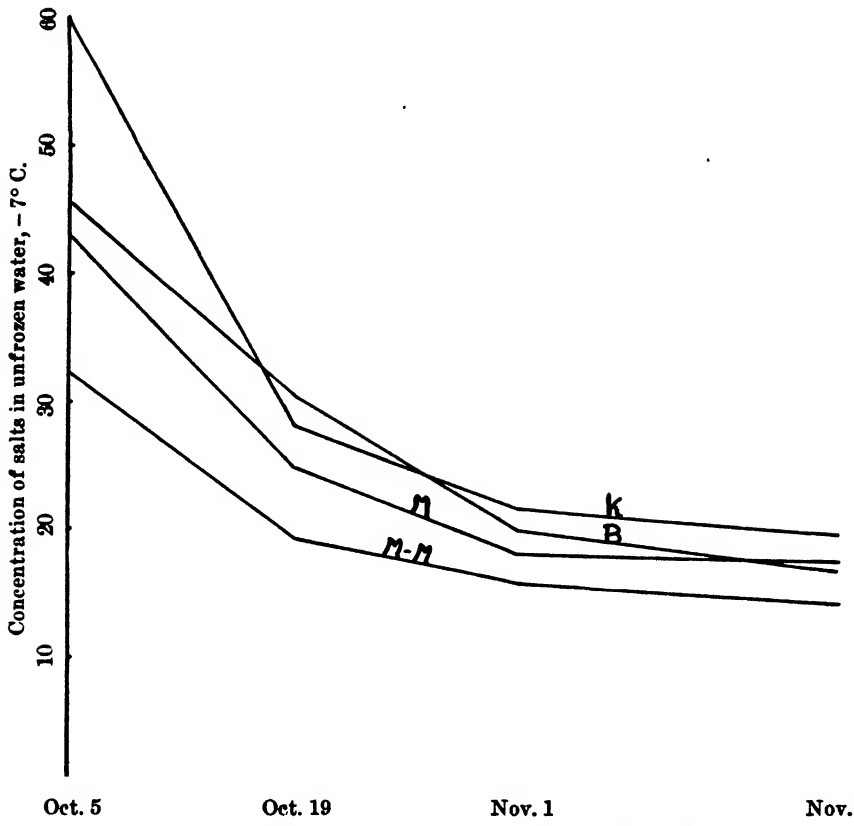


FIG. 4. Total extractable electrolytes of the sample (expressed as specific conductivity $\times 10^5$) have been divided by the values for grams-unfrozen-water to give the values on the vertical axis (– 7° C. series). Each curve shows the change in the electrolyte concentration in the unfrozen sap for a given variety as the season advanced. M-M(Minturki-Marquis) and M(Minturki) are hardier than K(Kanred) and B(Blackhull).

others have suggested that the injury to the protoplasm was due to the increase in salt concentration on freezing. The addition of sugars to plant saps or the accumulation of sugars within the plant has been repeatedly shown to favor winter hardiness (ÄKERMAN, 1) and to prevent coagulation of proteins in the frozen expressed juice (NEWTON and BROWN, 16). In either case, one evident effect of the added sugars would be correspondingly to decrease the concentration of salts in the sap at the freezing temperature used. The removal of electrolytes by diffusion, or by other methods, has not been shown, however, to cause any "protection" of the plant or the plant sap (NEWTON and BROWN, 16, DEXTER, unpublished data).

That the winter wheat plant becomes relatively low in soluble salts and

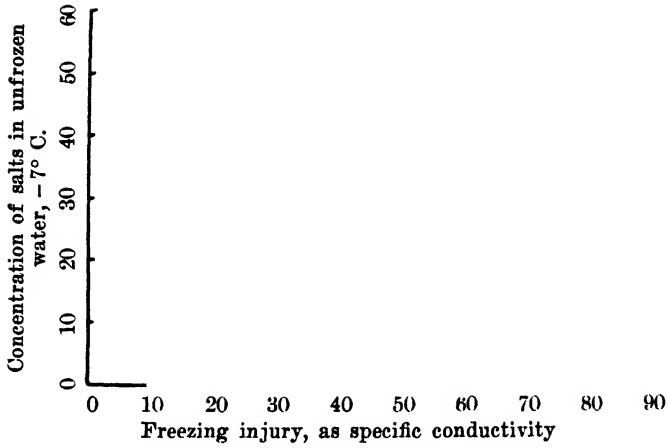


FIG. 5. Relationship between the concentration of salt in the unfrozen water in the plant and the injury due to freezing (as measured by exosmosis of salts, following freezing) is shown in the diagram. The points plotted are those given in table IV, for the – 7° C. series. High correlation between the two values is indicated by the almost linear form of the scatter-diagram.

relatively high in organic material as hardening proceeds in the field seems unquestionable from these data. The same decrease in soluble electrolytes was found, however, in plants grown in the greenhouse in sand cultures where a low concentration of mineral nutrients could not occur in the soil. Furthermore this decrease appeared to occur whether nitrogen was present in the cultures or not, and, in minus-nitrogen plants, took place in the dark, where hardening also occurred; but it did not take place in plus-nitrogen plants in the dark, where hardening failed to take place. Further studies are being made with greenhouse material.

In order to study further the relation of total minerals to hardiness as measured by the freezing-exosmosis method, measurements were made on 35 varieties of hybrid wheats which were seeded in six replicate rows on the University farm by the Agronomy Department. In the 35 varieties were included a few well-known varieties as indicators. Samples of crowns were used, as before, and weighed 5 grams. Percentage of dry matter was determined as previously stated. The samples were frozen in test-tubes; and exosmosis of mineral matter following freezing was measured in the usual way. The samples were taken, killed by heating, and extraction went on for 24 hours before measurements of total minerals were made. Determinations were made on three samples from each variety, all being obtained when the plants were in a fairly well hardened condition. The values for total extractable minerals and for freezing-exosmosis were averaged for the three samples. Figure 6 shows a scatter diagram of the relationship

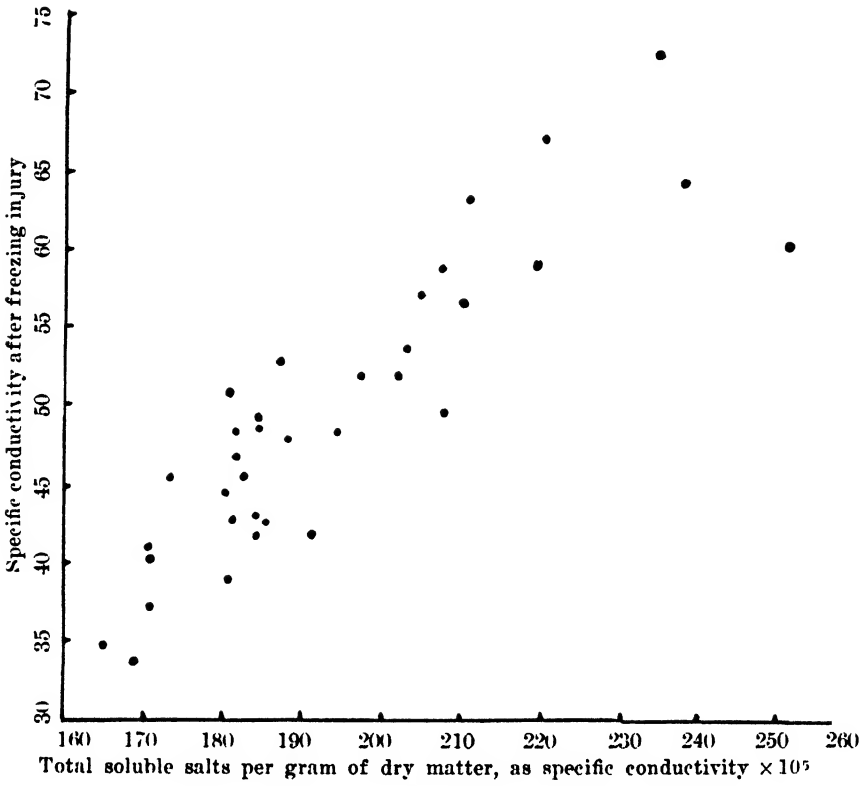


FIG. 6. Five-gram samples (triplicates) of crowns of 35 varieties of winter wheat were used. Exosmosis of salts into 25 cc. of water after freezing was measured conductimetrically to give the values on the vertical axis. The samples were then killed by heat. Diffusion of salts into the water continued for 24 hours when conductivity measurements were taken. These values, divided by the dry weight of the sample are shown on the horizontal axis.

between total extractable salts per gram of dry matter and conductivity resulting from exosmosis after freezing. The varieties of known hardiness fall, in this diagram, approximately where they would be expected, that is, hardiness was associated with low exosmosis of minerals following freezing, and with small amounts of soluble salt per gram of dry matter.

The figure indicates a considerable correlation between the values used. If extractable salts were plotted against percentage of dry matter, instead of against freezing-injury, a more or less similar diagram would result. That is, low percentage of dry matter is associated with high soluble mineral content and with low hardiness.

The total extractable salts in the samples of wheat crowns varied only perhaps 20 per cent., however, from the tenderest to the hardiest used in

this experiment. The extractable salts per gram of dry matter varied about 40 per cent. On the contrary, the salts extractable following freezing may vary several hundred per cent., depending upon the severity of freezing treatment used. Following very severe freezing treatment, extraction of salts is nearly the same as extraction following killing with heat, since differential freezing-injury is obscured by the heavy injury to hardy and tender varieties alike.

Discussion and summary

1. The data presented here show that withdrawal of water by ice-formation was not a fully reversible process in the wheat crowns used in this study. However, as the plants became more hardy, the process became more nearly reversible. This is in accord with observation of plants which have been frozen, either with or without injury. If they have been frozen beyond recovery, the water removed from the cells by ice-formation is not reabsorbed to give the previously turgid tissue.

2. Less water was left unfrozen in the tenderer varieties, but perhaps the difference is no more than would be expected on an osmotic basis from the slight differences in the freezing-point depression of the saps. The water left unfrozen at $-7°$ C. is, however, much greater than can possibly be explained on an osmotic basis from computations from the freezing-point depression of the boiled, expressed juice (LEBEDINCEV, **12**). At $-13°$ C. the excess unfrozen water is considerably smaller. That is, freezing at the lower temperature appeared to "unbind" the "bound" water (BRIGGS, **2, 3**).

3. As the season advanced and the plants became more hardy, the soluble mineral content of the tissues decreased in all four varieties. Since the dry matter in the samples increased, the soluble salt content per gram of dry matter decreased several fold. The concentration of soluble salts in the unfrozen water decreased very markedly, since the amount of unfrozen water tends to increase as hardiness increases. This was true with each variety as it hardened. It was also true that there was a higher salt concentration in the unfrozen water of the tender varieties than of the hardy ones.

4. In 35 winter wheat varieties studied, there was found a considerable inverse correlation between hardiness and soluble salts. In general, hardiness was associated with a high percentage of dry matter, and with low concentration of soluble salts.

Further studies are being carried on with other species of plants to determine whether these trends in mineral concentration are usually associated with the hardening process. An attempt is being made to determine what becomes of the soluble salts as hardening proceeds.

LITERATURE CITED

1. ÄKERMAN, Å. Studien über den Kaltetod und die Kalteresistenz der Pflanzen nebst Untersuchungen über die Winterfestigkeit des Weizens. Lund. pp. 1–232. 1927.
2. BRIGGS, D. R. Water relationships in colloids. I. Vapor-pressure measurements on elastic gels. Jour. Phys. Chem. **35**: 2914–2929. 1931.
3. ————. Water relationships in colloids. II. "Bound" water in colloids. Jour. Phys. Chem. **36**: 367–386. 1932.
4. CHANDLER, W. H. The killing of plant tissue by law temperature. Missouri Agr. Exp. Sta. Res. Bull. 8. 143–309. 1913.
5. DEXTER, S. T., TOTTINGHAM, W. E., and GRABER, L. F. Preliminary results in measuring the hardiness of plants. Plant Physiol. **5**: 215–223. 1930.
6. ————, ————, and ————. Investigations of the hardiness of plants by measurement of electrical conductivity. Plant Physiol. **7**: 63–78. 1932.
7. GORKE, H. Über chemische Vorgänge beim Erfrieren der Pflanzen. Landw. Vers.-Sta. **65**: 149–160. 1906.
8. GORTNER, R. A. The rôle of water in protoplasm. Ann. Rev. Biochem. **1**: 21–51. 1932.
9. HARVEY, R. B. The hardening process in plants and developments from frost injury. Jour. Agr. Res. **15**: 83–113. 1918.
10. HILL, A. V. Adventures in biophysics. University of Pennsylvania Press. 1931.
11. JONES, J. D., and GORTNER, R. A. Free and bound water in elastic and non-elastic gels. Jour. Phys. Chem. **36**: 387–426. 1932.
12. LEBEDINCEV, ELIZABETH. Untersuchungen über die wasserbindende Kraft der Pflanzen im Zusammenhang mit ihrer Dürre- und Kalteresistenz. Protoplasma **10**: 53–81. 1930.
13. MEYER, B. S. Further studies on cold resistance in evergreens, with special reference to the possible rôle of bound water. Unpublished manuscript.
14. MÜLLER-THURGAU, H. Über das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. **9**: 133–189. 1880.
15. NEWTON, R., and GORTNER, R. A. A method for estimating the hydrophilic colloid content of expressed tissue fluids. Bot. Gaz. **74**: 442–446. 1922.
16. ————, and BROWN, W. R. Frost precipitation of proteins. Canada Jour. Res. **5**: 87–110. 1931.
17. ROBINSON, W. Free and bound water determinations by the heat of fusion of ice method. Jour. Biol. Chem. **92**: 699–709. 1931.

18. ROSA, J. T. Investigations of the hardening process in vegetable plants. Missouri Agr. Exp. Sta. Res. Bull. 48. 1921.
19. SAYRE, J. D. Methods of determining bound water in plant tissue. Jour. Agr. Res. **44**: 669–688. 1932.
20. STEINMETZ, F. H. Winter hardiness in alfalfa varieties. Minnesota Agr. Exp. Sta. Tech. Bull. 38. 1926.
21. ST. JOHN, J. L. Temperature at which unbound water is completely frozen in a biocolloid. Jour. Amer. Chem. Soc. **53**: 4014–4019. 1931.

EFFECT OF SOIL TEMPERATURE ON TRANSPIRATION IN *HELIANTHUS ANNUUS*

F. E. CLEMENTS AND E. V. MARTIN

(WITH FOUR FIGURES)

Introduction

In the researches upon the adaptation of transplants to alpine and dune habitats, it has been essential to develop field methods for analyzing the factor complex. Chief among these are control batteries of phytometers in which one major factor is maintained in a series of intensities or levels while the others are held practically uniform, except for the daily march. Water content, light, wind, and soil temperature in particular permit such manipulation, and all are under investigation in the series of transplant gardens at Pike's Peak and at Santa Barbara. In both the alpine climax and the xerosere of litoral dunes, instrumentation has shown that wind and soil temperature are relatively extreme, and much attention has in consequence been directed to them.

The first experiments in this field were conducted by WHITFIELD (**12, 13**) at the Alpine Laboratory in 1930–1931 by means of standardized phytometers of *Helianthus annuus*, and the native *Mertensia sibirica*, which is much utilized in the transplant project. He demonstrated that a reduction of soil temperature from 113° to 51° F. lowered transpiration very little, while a decrease from 51° to 34° F. exerted a pronounced influence. Records of soil temperature during the growing season at 12,000 feet in the alpine tundra of Pike's Peak indicated a range from 35° to 48° F., with an average of 44° F. for the four years of observation (**14**).

A physiological test indoors of the relation between temperature and absorption was long ago made by SACHS (**7**), who placed a well-watered tobacco plant in a warm room and surrounded the pot with ice. After a brief period the plant began to wilt, but when the ice was removed and the soil heated, it recovered without the addition of water. By means of potometers, VESQUE (**10**) found that within the limits of 10° to 15° C. the absorption by roots increased rapidly as the temperature rises. KOZAROW (**3**) employed the same method to show that a fall from 20° to 0° C. retarded the rate of water supply by 25–30 per cent. Later investigations by DELF (**2**), STILES and JØRGENSEN (**9**), and by WEBER and HOHENEGGER (**11**) agree in demonstrating that the rate of absorption by a plant cell always increases with a rise of temperature, at least up to 30° C. Such responses in absorption are naturally reflected in transpiration, although STAHL (**8**) has discovered that temperatures near 0° C. may still permit a slight loss of water.

Methods

For the earlier series, plants of *Helianthus annuus* were grown from seed in water-tight, cylindric, galvanized iron cans 8″ in diameter and 10″ in height, while for later ones similar cans 8½″ × 11″ were employed. Each of these was fitted with a removable lid that had in its center a circular opening 2″ in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within 15 per cent. of this value throughout the growing period. When necessary, water was added through a glass tube extending into a layer of gravel about 1″ thick in the bottom of the can. When the plants were about six weeks old, the containers were sealed by adding a layer of sand about ½″ thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this type of seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that such containers seldom lost a detectable amount. This seal has the advantage over the paraffin type in that forced aeration is unnecessary (CLEMENTS and GOLDSMITH, 1).

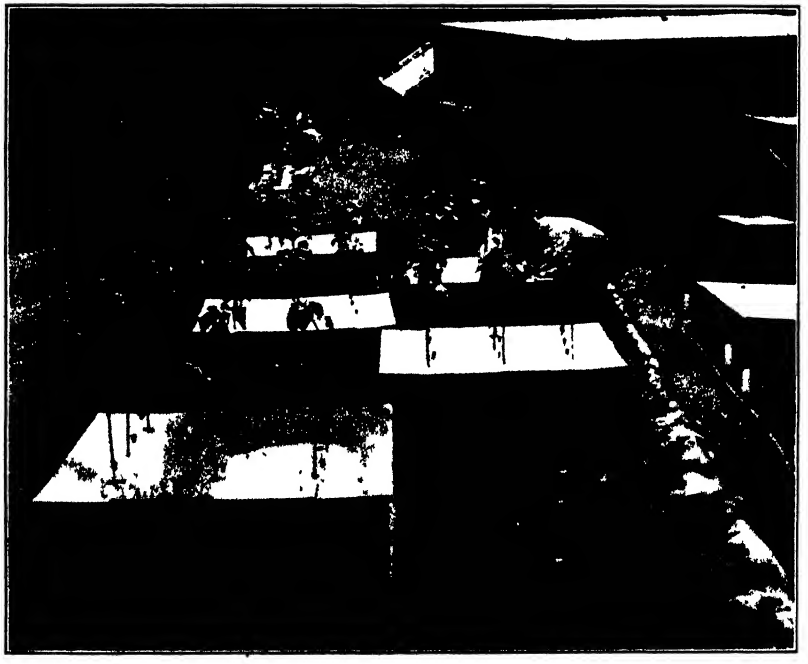


FIG. 1. Experimental installations as used in the later series, showing wilted condition of plants in a soil temperature of 30° F.

TABLE I

SOIL TEMPERATURE AND TRANSPIRATION

SERIES (1933)	AVERAGE SOIL TEMP.	INDIVIDUALS PER SET	TIME	TRANSPIRATION OF SET	SET LEAF AREA	TRANSPIRATION
	° *F.*		*hrs.*	*gm.*	*dm.²*	*gm./dm.²/hr.*
April 27	99	4	4.06	330	52.6	1.55
	73			295	53.1	1.37
	55			271	49.8	1.34
	46			254	54.5	1.15
	41			223	55.4	0.99
May 3	97	4	4.00	369	60.2	1.53
	74			339	51.5	1.65
	55			367	57.0	1.61
	47			318	53.9	1.47
	37			156	57.1	0.68
May 4	100	4	4.04	564	51.5	2.72
	75			485	60.2	1.99
	56			433	53.9	1.99
	48			348	57.1	1.51
	36			109	57.0	0.47
Aug. 25	99.5	9	4.31	379	60.3	1.46
	75			385	54.5	1.64
	55.5			374	58.0	1.50
	46			311	55.7	1.30
	36			143	58.8	0.56
Aug. 31	55.5	9	3.78	389	65.2	1.58
	51			382	62.9	1.61
	46			359	65.0	1.46
	40			272	69.0	1.04
	34			52	65.2	0.21
Sept. 2	52.5	9	4.09	835	68.3	2.98
	47			870	70.2	3.03
	42			752	72.3	2.54
	37.5			399	68.8	1.42
	34			138	68.8	0.49
Sept. 4	53	9	4.13	893	73.2	2.95
	47.5			910	72.5	3.04
	43			825	72.4	2.76
	38			562	69.6	1.81
	34.5			265	72.1	0.71

In the earlier series, five sets of four plants each were used, and these were placed in water baths 20″ square and 9″ deep, while in the later ones, five sets of nine plants each were employed with baths 3′ square and 10″ deep (fig. 1). In all cases these baths were held at fixed temperatures by additions of ice or hot water as occasion demanded, and any influence of the water on the immediate environment of the shoots was prevented by placing on the boxes closely fitting lids constructed of masonite or celotex.

On the day before a series was run, the phytometers were weighed and brought to their proper water content. At 6:00 A.M. the next day they were placed in the water baths, allowed four hours in which to become adjusted, and were then weighed to the nearest gram at 10:00 A.M. and again at 2:00 P.M. Soil temperatures were maintained constant throughout the series, and were registered by thermometers inserted near the centers of the cans. After the 2:00 P.M. weighing, the plants were taken out of the baths to permit recovery from any effects of the soil conditions, so as to be ready for future series. Leaf areas were determined in square decimeters by multiply-

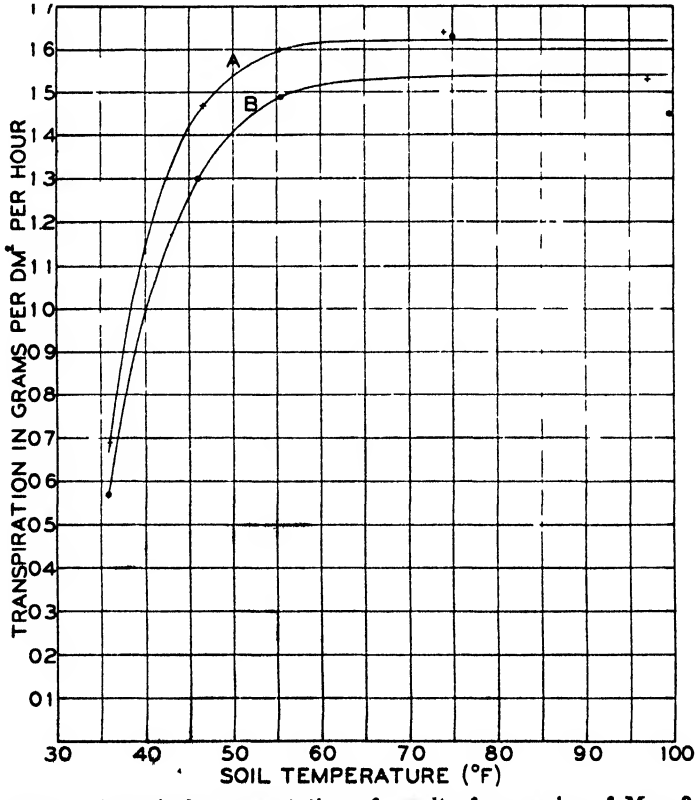


FIG. 2. Graphical representation of results from series of May 3 (A) and August 25 (B).

ing the product of length and width of the leaves in centimeters by the factor 0.013, according to the method of CLEMENTS and GOLDSMITH (1).

Results

In table I are given the data taken on seven different days, and in figure 2 the results for May 3 and August 25, 1933, are represented graphically

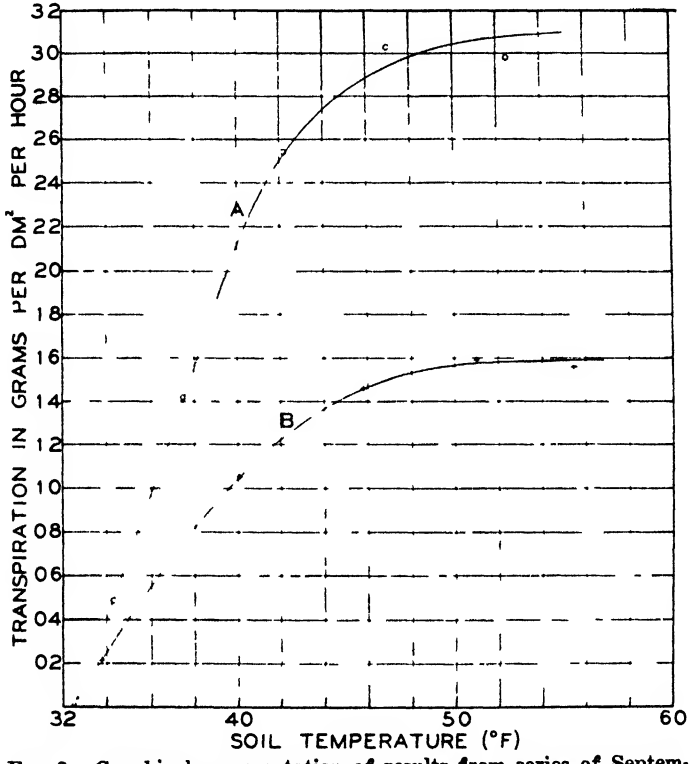


FIG. 3. Graphical representation of results from series of September 2 (A) and August 31 (B).

by curves A and B respectively, which portray transpiration in grams per square decimeter per hour (ordinate) as a function of soil temperature in degrees Fahrenheit (abscissa). Similarly, the data for September 2 and August 31, 1933, are represented in figure 3 by curves A and B respectively. The differences between the results for different days are due partly to the fact that the plants vary in age and partly to diversity in environmental factors (table II). Air temperature and relative humidity were measured at regular intervals during the various series, while the average wind velocity was obtained from the readings of a Weather Bureau four-cup anemometer. The light intensity was obtained from an estima-

TABLE II

METEOROLOGICAL DATA

DATE	AVERAGE AIR TEMPERATURE	AVERAGE RELATIVE HUMIDITY	AVERAGE WIND VELOCITY	LIGHT, ESTIMATED % FULL SUNLIGHT
	° *F.*	%	*m.p.h.*	%
April 27	65	49	1.9	25
May 3	73	24	3.5	100
May 4	80	27	4.1	100
Aug. 25	65	53	2.4	75
Aug. 31	66	45	2.0	50
Sept. 2	74	12	2.5	100
Sept. 4	75	21	2.1	100

tion of the relative lengths of time of cloudiness and full sunlight during the experiments.

The degree of opening of the stomata was secured by taking strips of lower epidermis of the leaves, fixing them in absolute alcohol, and observing them under a microscope (**6**). Since the stomatal aperture varied considerably within a narrow region of epidermis, only three degrees of opening are considered, namely, open, partially closed, and either narrow slits or closed; the results of these observations are given in table III. Since the condition as to wilting was closely connected with the degree of opening of the stomata, this also was noted and included in the same table. Under ordinary conditions, wilting begins with a soil temperature of about 40° and increases as the latter decreases to 32° F., while the stomata begin to close at about 40° and are nearly completely closed at 37°.

Accuracy of results

Errors in this type of experiment arise mainly from two sources, the experimental method and the differences between individual plants. The latter are caused principally by variations in growth or structure and differences in shading of lower leaves by the upper ones, while the former includes errors in the phytometer method, in leaf measurements, and in the weighing itself, as well as those arising from the length of time required to weigh the entire group of plants, which in these series amounted to about 25 minutes.

In order to discover the approximate values of the errors due to these various causes, 52 phytometers of *Helianthus annuus* were grown under similar conditions, both of soil and atmosphere, up to seven weeks of age. These were sealed as described and weighed at 10:00 A.M. and 2:00 P.M. on each of three successive days. The water content was maintained ap-

TABLE III

STOMATAL OPENING AND WILTING

DATE	TIME	AVERAGE SOIL TEMPERATURE					REMARKS ON WILTING
		99°	73°	55°	46°	41°	
April 27	10:00	Open	Open	Open	Open	P. Cl.*	Leaves of plants at 41° were a little less turgid than normally
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		97°	74°	55°	47°	37°	
May 3	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 37° somewhat wilted; edges of leaves curled a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		100°	75°	56°	48°	36°	
May 4	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 36° were wilted so that the crowns bent over a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		99.5°	75°	55.5°	46°	36°	
Aug. 25	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 36° were wilted so that the crowns bent over a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		55.5°	51°	46°	40°	34°	
Aug. 31	8:00	Open	Open	Open	Open	P. Cl.	Leaves of plants at 40° a little less turgid than normal; plants at 34° wilted so that crowns bent over considerably
	9:00	"	"	"	"	"	
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		52.5°	47°	42°	37.5°	34°	
Sept. 2		Strips unsatisfactory					Leaves of plants at 37.5° drooped and curled a little; plants at 34° wilted so that the crowns bent at nearly right angles to the main stem (fig. 1)

TABLE III—(*Continued*)

STOMATAL OPENING AND WILTING

DATE	TIME	AVERAGE SOIL TEMPERATURE					REMARKS ON WILTING
		53°	47.5°	43°	38°	34.5°	
Sept. 4	8:00	Open	Open	Open	Open	Open	Leaves of plants at 38° drooped and curled a little; plants at 34.5° wilted considerably, but crowns did not bend over
	9:00	"	"	"	"	P. Cl.	
	10:00	"	"	"	P. Cl.	Sl.-Cl.	
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	

* P. Cl., partially closed; Sl.-Cl., openings are either slits or are closed. Measurements of the stomatal openings for the earlier series were made by DR. F. L. LONG of the Carnegie Institution of Washington.

proximately constant by adding the proper amount each day. Leaf areas were measured on the first and third days, and the averages of these two were taken as the values for the intermediate day.

The average transpiration in grams per square decimeter per hour for the 4-hour period and the deviations of the individuals from this value were calculated. Since it required 30 minutes to weigh the 52 phytometers, the transpiration of the first plant weighed was measured for the period 10:00–2:00, while that of the last plant was from 10:30–2:30, the others falling between these two extremes. Since no drift in the deviations of the individuals was discernible, it was concluded that any error caused by lack of simultaneity of weighing was within the limits imposed by other sources. An approximate value of the error introduced by the latter was obtained in the following manner from the deviations of the individuals. The 52 phytometers were divided at random into 13 sets of four each, and the average transpiration for each set was determined for each of the three days. The maximum deviation of any one set from the average of the 13 sets for each day was 9 per cent., while the average deviation was 3 per cent. Similarly, when the 52 plants were grouped into sets of nine each, the maximum deviation observed was 4 per cent. and the average 1.5 per cent. It is probable, however, that the data obtained with the soil temperature experiments are not altogether as accurate as these figures indicate, since a slight error may arise from the effect of the water baths on the air about the plants, especially when the lids are lifted. In the measurement of temperature there may be appreciable errors due partially to gradients within the baths and partly to fluctuations about the average. Such deviations can only be estimated, but it is probable that the averages given in table I are in error by not more than about 1° F.

Discussion

In representing the data by smoothed curves as in figures 2 and 3, it is assumed that the relation between soil temperature and transpiration is a continuous one, and this assumption seems to be justified by the accuracy with which the curves fit the points. As can be seen from the curves, transpiration varies very little with soil temperatures between 55° and 100° F., but falls off below about 55° and is reduced to half at about 38°. Extrapolation of the curves in figure 3 indicates that transpiration approaches zero at 32.5°, although the accuracy of the data is not sufficient to fix this point at 32.5° and not 32° as is to be expected.

Obviously the explanation of this reduction in transpiration with soil temperatures below 55° F. is that the absorbing capacity of the root system is lowered, thus reducing the water supplied to the leaves. This usually does not produce a noticeable loss of turgor until the temperature has been decreased to about 40° F. The degree of wilting increases as the temperature is reduced below 40°, and at 34° has proceeded so far that the crowns of the plants are bent at nearly right angles to the main stems (table III). This wilting did not continue long enough to cause the plants any permanent injury, as was shown by the following test: On August 25, 1933, the set of plants at 36° F. (table I) was removed from its bath at 2:50 P.M. and placed in the 75° bath. This set and the one at 55.5° were weighed again at 4:00 P.M. and at 5:00 P.M. During the period from 2:50 to 4:00 the losses were 1.02 and 1.55 gm./dm.^{2}/hr. respectively, while during that from 4:00 to 5:00 the losses were 0.48 and 0.51 gm./dm.^{2}/hr. respectively. For this latter period the set that had wilted lost practically as much water as the set that had not. Also, by 4:00 P.M. the soil temperatures of both sets were about 55°, and none of the plants gave any visual signs of having been wilted.

The condition of the stomata during the various series is indicated in table III. Only three degrees of opening are considered, open, partially closed, and either narrow slits or closed, denoted in the table by the symbols Open, P.Cl., and Sl.–Cl., respectively. The stomata did not start to close until the soil temperature had been lowered to the point where the plants began to wilt perceptibly, about 40° F., but they were almost completely closed at 37°. These results indicate that it is not necessary for the stomata to close in order for transpiration to be reduced. For example, at 45° F. the transpiration was lowered appreciably, but the stomata showed no signs of closing; hence, the reduced water loss in this case must be due to a reduction in the water supplied to the leaves, which is in turn caused by a decrease in the absorbing capacity of the root system. Whether the amount of water transpired represents the amount absorbed during the same period of time is questionable, but since the degree of wilting of the

plants appeared to remain constant throughout the weighing period, it seems probable that such is the case.

KRAMER (**4**) points out that absorption of water by roots may be affected by temperature in two ways: first, by the physical effects (largely increased viscosity and decreased vapor pressure) which result in a slower movement of water from soil to root; and second, by the physiological effects on the permeability of the root cells. He gives data showing that the absorption of water from loam by soil-points (LIVINGSTON and KOKETSU, **5**) at 60° F. is approximately twice that at 32° F. Whether these results can be considered as a measure of the physical effects of low soil temperature on absorption of water by roots is questionable; but if so, then the results obtained with phytometers of *Helianthus annuus* indicate that the physiological effects are of greater importance than the physical.

The data presented here agree with those of the earlier workers already mentioned, and they also constitute an addition to present knowledge in that the effect upon transpiration of soil temperature has been traced through the critical region 34° to 55° F. So far as is known, this range has not heretofore been investigated.

The applicability of these results to alpine problems is definitely limited in that the plants were grown under soil temperatures of 60°–70° F., and the effect of lower soil temperatures upon transpiration has been measured for only the first few hours after the plants were subjected to those conditions. However, since WHITFIELD (**12**) has shown that transpiration of phytometers of *Helianthus annuus* in alpine regions (12,000 feet on Pike's Peak) may be as much as 2 gm./dm².hr., the results of curve B of figure 3 are applicable to this climatic region. Soil temperatures in this same climate are represented graphically in figure 4 (taken from WHITFIELD, **14**),

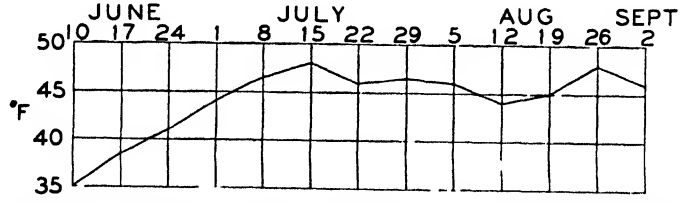


FIG. 4. Average soil temperature at 4 inches, by weeks 1927–1930, at 12,000 feet on Pike's Peak (from WHITFIELD, 1933).

which shows that there is a variation between 35° and 48° F. for the growing season. Accordingly our results indicate that, in this particular alpine climate at least, soil temperature probably has an appreciable influence on transpiration and growth of vegetation. These results are obtained with phytometers of *Helianthus annuus*, however, and it is possible that native alpine plants would respond differently.

The final test of this influence remains to be carried out. Experiments are planned in which plants will be grown in the field from seeds or seedlings under various soil temperatures with other environmental factors similar, and the growth, transpiration, and other functional activities will be measured. This problem will require several thermostatically controlled baths, but it is expected to yield considerable information concerning the effect of low soil temperature on vegetation.

Summary

1. The effect of soil temperatures in the range 34°–100° F. on transpiration of *Helianthus annuus* phytometers 6–8 weeks old was measured by using five sets of four or nine plants each, run simultaneously in water baths of different temperatures.

2. It was found that transpiration varies very little with soil temperatures between 55° and 100°, but drops rapidly below 55°, was reduced to half at 38°, and approached zero at 32°.

3. The temperature of the baths was held constant within 1° F., and some data are presented that indicate an accuracy in transpiration measurements of from 3 to 5 per cent.

4. Strips of lower epidermis of the leaves show that the stomata do not begin to close until the soil temperature is lowered to about 40°, but are nearly completely closed at 37° F.

5. The plants usually start to wilt at about 40° and at 34° are wilted so that the crowns are bent at nearly right angles to the main stems.

6. The plants recover rapidly from the wilted condition when the soil temperature is raised.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

LITERATURE CITED

1. CLEMENTS, F. E., and GOLDSMITH, G. W. The phytometer method in ecology. Carnegie Inst. Washington Publ. No. 356. 1924.
2. DELF, M. Studies of protoplasmic permeability by measurement of rate of shrinkage of turgid tissues. I. The influence of temperature on the permeability of protoplasm to water. Ann. Bot. **30**: 283–310. 1916.
3. KOZAROW, P. Einfluss verschiedener äusserer Faktoren auf die Wasseraufnahme der Pflanzen. Dissert., Leipzig. 1897.
4. KRAMER, P. J. Effects of soil temperature on the absorption of water by plants. Science **79**: 371–372. 1934.
5. LIVINGSTON, B. E., and KOKETSU, R. The water-supplying power of

the soil as related to the wilting of plants. Soil Sci. **9**: 469–485. 1920.

6. LLOYD, F. E. The physiology of stomata. Carnegie Inst. Washington Publ. No. 82. 1908.
7. SACHS, J. Beiträge zur Lehre von der Transpiration der Gewächse. Bot. Zeit. **18**: 121–126. 1860.
8. STAHL, E. Zur Physiologie und Biologie der Exkrete. Flora **113**: 1–132. 1919.
9. STILES, W., and JÖRGENSEN, I. Studies in permeability. V. The swelling of plant tissues in water and its relation to temperature and various dissolved substances. Ann. Bot. **31**: 415–434. 1917.
10. VESQUE, J. De l'influence de la température du sol sur l'absorption de l'eau par les racines. Ann. Sci. Nat. Bot. **6**: 169–209. 1878.
11. WEBER, F., and HOHENEGGER, H. Reversible Viscositätserhöhung des Protoplasmas durch Kälte. Ber. deut. bot. Ges. **41**: 198–205. 1923.
12. WHITFIELD, C. J. Ecological aspects of transpiration. I. Pike's Peak region; climatic aspects. Bot. Gaz. **93**: 436–452. 1932.
13. ———. Ecological aspects of transpiration. II. Pike's Peak and Santa Barbara regions; climatic and edaphic aspects. Bot. Gaz. **94**: 183–196. 1932.
14. ———. The vegetation of the Pike's Peak region. Ecol. Mon. **3**: 75–105. 1933.

RELATION OF BENZOIC ACID CONTENT AND OTHER CONSTITUENTS OF CRANBERRIES TO KEEPING QUALITY[1]

J. A. Clague and C. R. Fellers

Introduction

The benzoic acid naturally occurring in cranberries has long been regarded as the preserving agent responsible for the good keeping qualities of the berries and the products manufactured from them (**10, 15**). The purpose of this investigation was to determine the benzoic acid content of several Massachusetts, New Jersey, and Wisconsin varieties, and to note the amount present in relation to other constituents and to the keeping qualities of the berries.

Loew (**8**) was probably the first to report the presence of benzoic acid in the German Preisselbeere (*Vaccinium vitis idaea*). Subsequent quantitative determinations were reported by other workers (**5, 7, 9**). Griebel (**4**) thoroughly studied the free and combined benzoic acid of the Preisselbeere, the Moosbeere (*V. oxycoccus*), and the American cranberry (*V. macrocarpum*). He found the Preisselbeere to contain as much as 0.22 per cent. total benzoic acid, the Moosbeere and the cranberry having a content of 0.02–0.06 per cent. Mason (**10**) found the American cranberry to contain 0.05 per cent. benzoic acid; Radin (**16**) reports 0.06 per cent. as the content; Blatherwick and Long (**2**) give the percentage as 0.096; and Nelson (**14**) reports 0.069 per cent. The varieties analyzed were not stated in the papers. Chemical composition of the important American varieties of cranberries has been studied by Morse (**12**).

Experimental methods

The cranberries were shipped to the laboratory in quarter-barrel boxes and were put into cold storage (2° C.) upon receipt, samples being taken out as needed for analysis.

The procedure followed in the determination of benzoic acid was for the most part that recommended by the A. O. A. C. (**1**). A modification was made in the treatment of the chloroform extract. The residue from the latter was dissolved in ether and transferred to test tubes and treated in the manner described by Monier-Williams (**11**). The ether was evaporated under a stream of air dried by bubbling through concentrated sulphuric acid. The residue was washed down from the sides of the tubes until it was contained in the bottom 2 or 3 cc. Some previously ignited sand was added and a mark filed on the test tubes about 4 cm. from the

[1] Contribution No. 195 of the Massachusetts Agricultural Experiment Station.

bottom. A disk of filter paper was pushed down to the mark. A 1-gallon slip cover can was used as a sublimator. The test tubes were pushed through holes in an asbestos mat which fitted over the can, the test tube base resting on a wire basket inside the can, so that the filed mark was just below the surface of the asbestos mat. Cork stoppers were placed loosely over the mouth of the test tubes and they were heated at 160°–170° C. for about two hours. After cooling, the bottom 4 cm. of the tubes were cut off at the mark. The crystals of benzoic acid were all sublimated in the upper part of the tube. Any that had fallen down were caught on the filter paper disc, which was removed and from which the crystals were shaken back into the upper part of the test tube. The test tubes with the sublimate were dried in a desiccator over sulphuric acid and then weighed. The benzoic acid was washed into a small Erlenmeyer flask with alcohol, and the test tubes again weighed. The difference in weight represents the benzoic acid present in the sample. The sublimate washed out of the test tube was titrated with 0.05 N sodium hydroxide, thus giving a check on the gravimetric determination. This modified method gave very reliable results and reduced the time required to make a determination.

Total acids and pectin, as alcohol precipitate, were determined by the A. O. A. C. (1) methods.

The soluble solids content of the juice from new crushed cranberries was determined with the Abbé refractometer. Results are not so accurate as those obtained by gravimetric methods, but give good comparative data.

The number of berries per quart container was considered to be the most convenient measure for their size. The weight of a quart of berries represents the average weight of ten quarts.

Results

The benzoic acid content of 24 varieties is shown in table I. Results for two years are given for four varieties. The percentage of the berries spoiled at the time of analysis is shown. The rating of varietal keeping qualities as given by C. S. BECKWITH of the New Jersey Cranberry Station, H. J. FRANKLIN of the Massachusetts Station, and C. M. CHANEY of the American Cranberry Exchange is also shown.

With one exception (Berry Berry variety) the percentage of benzoic acid was over 0.05, the maximum being 0.098.

According to CRUESS and IRISH (3), fruit products with a pH of 3 to 3.5 require less than 0.05 per cent. sodium benzoate (equivalent to about 0.04 per cent. benzoic acid) for preservation. In grape products, where the pH was 2.6 and 3.0, the equivalent of 0.02 per cent. benzoic acid sufficed to preserve the fruit against yeast spoilage.

The pH of sound cranberries is 2.35–2.6 (17); hence preservative con-

TABLE I

VARIETAL DIFFERENCES IN CHEMICAL COMPOSITION AND KEEPING QUALITY

VARIETY	SOURCE	BERRIES PER QUART	WEIGHT PER QUART	ESTIMATED KEEPING QUALITY	DECAYED AFTER 4 MONTHS AT 35° F.	BENZOIC ACID		TOTAL ACID	PECTIN AS ALCOHOL PRECIPITATE	SOLUBLE SOLIDS BY REFRACTOMETER
						1931–32	1932–33			
			oz.		%	%	%	%	%	%
Berry Berry	Massachusetts	237	13.2	Poor	43	0.029		2.08	1.06	8.2
Bugle		343	12.8	Good–excellent	18	0.068		2.77	1.29	8.6
Centennial		239	12.6	Poor–fair	80	0.062		2.55	1.18	9.2
Early Black		408	12.9	Fair–very good	80 (soft)	0.055	0.071	2.51	0.91	7.2
Holliston		238	11.8	Poor	30	0.054		2.29	1.4	8.0
Howes		358	13.7	Very good	15 (soft)	0.072	0.08	2.27	1.24	9.2
Maxim Randall		320	12.7	Good–very good	16	0.063	0.084	2.46	1.14	8.2
Maxim Randall (Admixture)		297	12.8	Good			0.067	2.46	0.90	9.2
Shaw's Success		396	14.0	Good–very good	6	0.077	0.098	2.55	1.17	9.2
Shurtliff		295	12.2	Fair	50	0.077		2.38	1.22	8.8
Smalley Howes		423	13.0	Good	6	0.076		2.80	1.13	8.6
Tom Howes		324	12.8	Fair			0.072	2.27	0.86	9.6
Braddock Bell	New Jersey	322	12.7	Good	24	0.080		2.25	1.03	9.8
Budd's Blues		600	13.4	Excellent	10	0.066		2.17	1.23	10.6
Early Richards		360	12.8	Poor–fair	35	0.091		2.56	1.08	9.2
Harold		325	12.5	Excellent	25	0.053				
Howard Bell		316	12.8	Fair	25	0.079		2.22	1.15	9.6
Nancy Munyon		508	12.7	Fair	50	0.074		2.20	1.10	8.8
Plum		450	14.3	Good–very good	31	0.083		2.31	1.66	8.6
Woolman's		327	12.3	Good	17	0.073		2.19	0.95	9.8
Bennett's Jumbo	Wisconsin	333	12.7	Good–very good	12	0.068		2.68	1.36	9.6
Berlin		463	13.6	Good–very good	37	0.077		2.43	1.30	9.2
Gebhardt's Beauty		356	13.0	Good	50	0.088		2.46	1.41	9.8
Searls Jumbo		395	13.1	Poor–fair	40	0.077		2.28	1.04	9.8
Average		360	12.9		32	0.065	0.079	2.35	1.16	9.1

ditions in cranberries are apparently ideal. However, annual loss from fruit rot does constitute as much as 25 per cent. of the total cranberry crop of the United States (**18**).

There seems to be no definite relationship between the keeping quality of cranberries and their benzoic acid content. Factors other than the latter must be considered. The infection by spoilage microorganisms usually occurs relatively early in the growing season (**19**). The benzoic acid is present only in traces in the immature berries and the amount increases gradually to a maximum as the berry ripens (**4, 10**). Benzoic acid exerts a preservative rather than a germicidal action on microorganisms, in the concentrations in which it is present in the ripe cranberry. If the infective agent has already established itself, therefore, the gradually increasing quantities of the acid apparently are not sufficient to destroy the fungi.

Furthermore, GRIEBEL (**4**) has pointed out that some of the benzoic acid in cranberries is combined as esters and as a glucoside of vacciniin. As much as 35 per cent. of the total benzoic acid was in the form of the glucoside in some American cranberries analyzed by GRIEBEL. It is not known whether this combined form of benzoic acid exerts any antiseptic action.

The percentage of total acids (calculated as citric) in the cranberries analyzed varied from 2.08–2.80, with an average of 2.35. Of the nine varieties listed as only fair or as poor keepers, six had total acid contents below the average and one was only 0.03 per cent. above. The keeping qualities of 15 varieties were rated from good to excellent. Nine of these had total acid contents above and six below the average.

Pectin as alcohol precipitate was found to vary between 0.86 and 1.66 per cent., averaging 1.16 per cent. No definite relationship existed between keeping quality and pectin content. The average soluble solids content was 9.1 per cent., with a maximum of 10.6 and a minimum of 7.2.

Some of the best keeping varieties had a high soluble solids content, the variety Budd's Blues having the maximum amount and having a rating as excellent. On the other hand, some of the berries which were poor keepers also had a high percentage of soluble solids.

EFFECT OF QUINIC ACID AS A PRESERVING AGENT

Cranberries are known to have a quinic acid content of as much as 1 per cent. (**6, 13**). The following test was made to determine the efficiency of quinic acid as an antiseptic, and also to note the effect of the combination of quinic and benzoic acids.

To one series of tubes containing sterile sweet cider there was added 2 per cent. quinic acid plus 0.1, 0.05, and 0.025 per cent., and no sodium benzoate. A similar series was made with the addition of 1 per cent. quinic acid and a third contained the sodium benzoate alone. The media were

inoculated with yeasts and molds and incubated at room temperature. Results are shown on table II.

TABLE II

PRESERVATIVE EFFECT OF QUINIC AND BENZOIC ACIDS

Quinic acid per cent.	2	2	2	2	1	1	1	1	0	0	0
Benzoic acid per cent.	0.1	0.05	0.025	0	0.1	0.05	0.025	0	0.1	0.05	0.025
Yeasts	−	−	−	+	−	−	−	+	−	−	+
Molds	−	−	−	+	−	−	−	+	−	−	−

After three days' incubation there was abundant growth in all tubes containing the quinic acid alone, and no growth in the others. At the end of ten days the only change was that yeast was growing in the tube containing 0.025 per cent. sodium benzoate alone. The increased acidity of the media containing the added quinic acid probably accounted for this difference. No further changes were noted after a month's incubation period. It was concluded that the naturally occurring quinic acid did not exert an appreciable antiseptic or germicidal action on fruit spoilage organisms.

Summary and conclusion

1. The benzoic acid content of twenty-four varieties of cranberries ranged from 0.029 to 0.098 per cent., with an average of 0.065 per cent.

2. The varieties with the best keeping qualities did not always have a high benzoic acid content, so that apparently factors other than benzoic acid content alone must be largely responsible for the keeping properties of the berries.

3. The quinic acid present did not exert any appreciable preservative action on spoilage organisms.

4. The large percentage of varieties having poor keeping qualities had low total acid content, while the good keeping varieties in the majority of cases had a high total acid value.

5. Pectin and soluble solids content did not correlate with keeping quality.

6. Benzoic acid is present in the ripe berry in amounts sufficient to give a preservative action. However, high benzoic acid content in a variety is not always indicative of good keeping quality; apparently certain physical or environmental factors are of greater importance.

MASSACHUSETTS STATE COLLEGE
AMHERST, MASSACHUSETTS

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 2d ed. 1925.

2. BLATHERWICK, N. R., and LONG, M. L. The increased acidity produced by eating prunes and cranberries. Jour. Biol. Chem. **57**: 815–818. 1923.
3. CRUESS, W. V., and IRISH, J. H. Rôle of acidity in the preservation of fruit specialties. Fruit Products Jour. and Amer. Vinegar Ind. p. 177. Feb. 1931.
4. GRIEBEL, C. Beiträge zur Kenntnis der chemischen Zusammensetzung der Preisselbeeren, Moosbeeren und Kranbeeren. Zeitschr. Untersuch. Nahr. und Genussmtl. **19**: 241–252. 1910.
5. KANGER, Zur Frage über die chemische Zusammensetzung und die pharmakologische Wirkung der Preisselbeeren. Arch. experim. Pathol. u. Pharmakol. **50**: 46–75. 1903.
6. KOHMAN, E. F., and SANBORN, N. H. Isolation of quinic acid from fruits. Ind. & Eng. Chem. **23**: 126. 1931.
7. LEHMANN, K. B. Die Benzoesäure. Chem. Zeitung. **32**: 949. 1908.
8. LOEW, O. Über die Quelle der Hippursäure im Harn der Pflanzenfresser. Jour. Prakt. Chem. **19**: 309–312. 1879.
9. MACH, E., and PORTELE, K. Über die schwere Vergärbarkeit und die Zusammensetzung des Preisselbeersaftes. Landw. Vers.-Sta. **38**: 69–78. 1890.
10. MASON, G. C. The occurrence of benzoic acid naturally in cranberries. Jour. Amer. Chem. Soc. **27**: 613–614. 1905.
11. MONIER-WILLIAMS, G. W. Determination of benzoic acid in foodstuffs. Analyst **52**: 229. 1927.
12. MORSE, F. W. A chemical study of cranberries. Massachusetts Agr. Exp. Sta. Bull. 265. 1930.
13. ————————. Annual report, Massachusetts Agr. Exp. Sta. Bull. 280. 1932.
14. NELSON, E. K. Non-volatile acids of the pear, quince, apple, loganberry, blueberry, cranberry, lemon and pomegranate. Jour. Amer. Chem. Soc. **49**: 1300–1302. 1927.
15. NESTLER, A. Ein Schutzmittel der Preisselbeere. Die Umschau. **13**: 1016–1018. 1909.
16. RADIN, M. J. A note on the quantity of benzoic acid contained in prunes and cranberries. Ind. & Eng. Chem. **6**: 518. 1914.
17. RICE, C. C. Preservation, utilization and properties of cranberry juice. Master's thesis. Massachusetts State College. 1932.
18. SHEAR, C. L. Cranberry diseases and their control. U. S. Dept. Agr. Farmers Bull. 1081. 1920.
19. STEVENS, N. E. Notes on cranberry fungi in Massachusetts. Phytopath. **14**: 101–107. 1924.

EFFECT OF ETHYLENE, ETHYLENE CHLOROHYDRIN, AND ULTRA-VIOLET LIGHT ON CARBOHYDRATE CONTENT OF STORED APPLES[1]

ROBERT B. DUSTMAN

Literature review

In 1924 CHACE and DENNY (2) described the use of ethylene as a new and satisfactory method for the coloring of citrus fruit. Since that time various investigators have reported the effect of this and other substances upon the general ripening processes of fruits and vegetables. Differences of opinion have arisen concerning the effect of these ripening agents upon the color changes and the chemical composition of the treated fruits and vegetables. REGEIMBAL and HARVEY (9) found a decrease in total sugars and an increase in direct-reducing sugars in pineapples treated with ethylene or propylene. They suggested that the increase of direct-reducing sugars arises from sucrose and concluded that the activity of the proteolytic enzymes and invertase of pineapples is increased by treatment with ethylene or propylene. ENGLIS and ZANNIS (3), however, reported no acceleration of taka-diastase action on soluble starch when the solutions were treated with ethylene. This was true also for the diastase of corn meal in acting upon the starch of the grain. The rate of hydrolysis of sucrose by invertase likewise showed no increase for ethylene treatment.

In the case of celery blanched with ethylene, HARVEY (4) states that "by taste and by chemical analysis treated celery was found to be higher in sugar content than untreated celery. It was found, also, that tomatoes treated with ethylene were sweeter than untreated fruits." With tomatoes he reported a frequent initial appearance of the red color at the stem end with subsequent spreading from this region in ethylene-treated fruits. KOHMAN (6), however, could not confirm this color behavior but reported that, while color development might be hastened with ethylene, it proceeded in the normal manner from the blossom end. Also, HIBBARD (5) found that although ethylene destroys chlorophyll and thus blanches celery, chemical studies show that ethylene-treated celery is not sweeter than celery blanched by boards, and the hearts and stalks of ethylene-treated celery contain less total sugars than check lots. With tomatoes, chemical analyses showed a much higher total sugar content for vine-ripened fruits than for those artificially ripened, but a similar comparison between ethylene-ripened and air-ripened tomatoes gave no significant difference. This is said to be at

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 126.

variance with ROSA's (10) findings that ethylene-treated tomatoes are sweeter than those air-ripened. Later ROSA (11) reported no increase in sugar content of Honey Dew and Casaba melons picked slightly unripe and exposed to ethylene treatment, but the treated melons did show a marked acceleration in the rate of softening, in change of green to yellow color of the rind and in conversion of reducing sugars to sucrose.

CHACE and CHURCH (1) obtained no appreciable alteration of the composition of the edible portion of citrus fruits treated with ethylene, but did find an acceleration in the color development. The same treatment hastened the coloring of persimmons, destroying the astringency and producing softening.

RAMSAY and MUSSO (8) state that in experiments with oranges, total sugar content was not increased by treating the fruit with ethylene but actually was slightly diminished. They report also "an increase in fruit sugar, however, from the hydrolization of the cane sugar present—a process the reverse of ripening."

WOLFE (12) studied the effect of ethylene on the ripening of bananas and found only a very slight increase in sugars and decrease in starch, from day to day, resulting from the ethylene treatment. The treated fruit turned yellow at a somewhat more rapid rate than did the controls.

HARVEY (4) states that "when green apples are intended for cider or vinegar it will probably be advantageous to sweeten them by the use of ethylene. It should be remembered that starches are rather quickly digested under the ethylene treatment, so that better quality in the cider may be produced by the increase in sugars as well as by the removal of the excess fruit acids." No data on composition are given.

HIBBARD (5) reports an experiment in which ethylene was used on McIntosh apples in order to study its effect on color. The apples were large and of fancy grade except for lack of uniform color. They were given daily treatments of ethylene over a 2-weeks' period. The treatment had no effect on the development of red color but did destroy the green in the green colored areas, leaving them yellow. No analyses are reported.

The investigation about to be described was carried out for the purpose of obtaining data on the effect of ethylene on the composition of stored apples, special attention being given to the carbohydrate group. Treatment with ethylene chlorohydrin and irradiation with ultraviolet rays were also included for purposes of comparison.

Materials and methods

In October, two bushels of each variety of apple were picked from trees of Stayman Winesap, Rome Beauty, and Ben Davis. Each variety was picked from a single tree only and removed to cold storage (33° to 36° F.)

within 4 hours after picking. The fruits when taken from the trees were selected for uniformity of size, firmness, color, and maturity. On March 6, four lots were chosen from each variety, each lot consisting of five apples. Again at this time every effort was made to select uniform sound fruits, the general condition of all three varieties being excellent.

Investigation

TREATMENT

The various lot treatments for each variety were given daily (except Sundays) as follows:

Ethylene, 1 to 1000 by volume; ethylene chlorohydrin, 1 cc. of 40 per cent. solution per bottle (allowed to evaporate); irradiation with a universal model Hanovia mercury arc lamp for 60 minutes (30 minutes on each side) at 30-cm. distance. The lots were brought from cold storage each day except Sundays, the containers thoroughly irrigated with fresh air, the treatments repeated, and the fruits returned to storage within 1½ to 2 hours. Control lots were carried to and from storage, irrigated, removed from their containers, exposed to room temperatures during the same periods, replaced, and otherwise handled as nearly like the treated lots as could be done.

The containers used were 7-liter, wide-mouthed glass bottles equipped with rubber stoppers. The actual number of daily treatments given was 35, extending over a period of 41 days. At the end of this period the cores of all fruits were removed with a cork borer, and diagonal quarters taken from each of the five apples in each lot for chemical analysis. At the same time the half-apple remnants were used for the immediate determination of pH of the expressed juices. This was accomplished by using a Leeds and Northrup Type K potentiometer with a quinhydrone electrode, after squeezing out the juice rapidly through muslin cloth in a small hand-press.

CHEMICAL ANALYSES[2]

The samples taken for analysis were preserved in the usual manner. They were weighed, sliced rapidly, and stored after heating in approximately 80 per cent. alcohol in glass bottles. On removal from storage they were evaporated to dryness in open dishes and dried in a vacuum oven for 48 hours at 65° C. and at a pressure 27 to 27½ inches of mercury below atmospheric. After drying, the samples were ground in an agate mortar by hand.

Extraction was carried out first with ether and then with 80 per cent. alcohol, using Soxhlet extractors with glass thimbles and ground glass joints. The total extraction period for each solvent was 24 hours.

[2] Acknowledgment is gratefully made to Mr. L. C. SHRIVER, former Assistant in Agricultural Chemistry, for much of the chemical work herein reported.

The alcoholic extracts were evaporated in the usual manner and the sugar solutions cleared with neutral lead acetate and potassium oxalate.

Reducing sugars were determined directly on an aliquot of the cleared solution, using Soxhlet's modification of Fehling's solution, and the results calculated as invert sugar.

Total sugars were similarly determined after hydrolysis with HCl at 37.5° C. (A.O.A.C. method, 7). The difference between the total and the reducing sugars was multiplied by the factor 0.95 and designated as sucrose.

Starch was determined by gelatinizing the residues from the alcoholic extractions with hot water, digestion with taka-diastase at 37.5° C. for 24 hours, filtration, and subsequent hydrolysis with HCl. The reducing values were calculated as glucose. This value multiplied by the factor 0.90 was designated as starch.

Hemicellulose was determined on the residues from the starch determination. These were treated with 1 per cent. HCl on the boiling water bath for three hours. During this treatment the flasks were equipped with reflux condensers and completely immersed. Following hydrolysis, the filtrates were cooled, neutralized, transferred, made up to volume, and the reducing power calculated as glucose.

Results

The effect of the treatments described on the chemical composition of the fruits is shown in table I and II.

TABLE I

FRACTIONAL COMPOSITION AND pH VALUES OF APPLES VARIOUSLY TREATED

VARIETY	TREATMENT	TOTAL MOISTURE	pH OF EXPRESSED JUICE	MOISTURE-FREE BASIS	
				ETHER EXTRACT	SOLUBLE IN 80 PER CENT. ALCOHOL
		%	*pH*	%	%
Ben Davis	None	84.9	3.59	5.93	72.40
	Ethylene	84.6	3.53	6.17	72.67
	Ethylene chlorohydrin	84.2	3.67	5.78	73.72
	Ultraviolet	85.2	3.57	5.05	73.69
Stayman Winesap	None	81.7	3.38	5.26	79.65
	Ethylene	81.3	3.32	5.45	79.65
	Ethylene chlorohydrin	82.0	3.44	3.58	81.04
	Ultraviolet	82.1	3.43	3.90	81.04
Rome Beauty	None	86.4	3.43	5.29	77.31
	Ethylene	86.6	3.60	3.34	79.42
	Ethylene chlorohydrin	87.5	3.55	3.84	78.97
	Ultraviolet	86.2	3.58	3.78	78.81

TABLE II

CARBOHYDRATE CONTENT OF APPLES VARIOUSLY TREATED; MOISTURE-FREE BASIS

VARIETY	TREATMENT	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE (0.95 × DIFFERENCE)	STARCH (0.90 × GLUCOSE VALUE)	HEMICELLULOSE AS GLUCOSE
		%	%	%	%	%
Ben Davis	None	47.94	61.85	13.21	0.48	2.89
	Ethylene	49.72	62.58	12.22	0.51	2.22
	Ethylene chlorohydrin	50.80	63.01	11.60	0.59	2.20
	Ultraviolet	49.85	63.92	13.37	0.51	2.12
Stayman Winesap	None	57.31	71.07	13.07	0.31	1.07
	Ethylene	57.26	72.16	14.16	0.52	0.80
	Ethylene chlorohydrin	58.88	70.09	10.65	0.81	1.25
	Ultraviolet	57.64	71.48	13.15	0.61	1.41
Rome Beauty	None	57.10	71.15	13.25	0.43	0.85
	Ethylene	57.64	71.55	13.21	0.55	1.17
	Ethylene chlorohydrin	57.77	72.64	14.13	0.62	0.99
	Ultraviolet	58.02	73.39	14.60	0.53	0.91

After about four weeks, the ethylene-treated fruits were beginning to show a more rapid change of ground color from green to yellow than the untreated fruits. By the close of the experiment this color change was quite noticeable, and in addition a considerable mellowing and softening effect was apparent when the fruits were sampled for chemical analysis.

By the last days of March some of the Ben Davis apples treated with ethylene chlorohydrin were showing slight signs of scald, but this did not become pronounced during the remaining days of the experiment. The Stayman and Rome varieties were not so affected, but all three varieties under ethylene-chlorohydrin treatment showed some softening as compared with the controls.

The only noticeable effect of the ultra-violet irradiation was a very slight bronzing of the most directly exposed portions of the fruits, where the color was green.

At the close of the experiment the untreated apples of all three varieties were firm, sound, and practically unchanged in ground color.

Discussion

Examination of the values in both tables reveals a remarkably close agreement in the results from the variously treated and untreated lots. This is true even for such complex groups as the alcohol-soluble and hemicellulose fractions. The values for water content are likewise uniform for each variety. Collectively considered this uniformity of composition in treated and untreated fruits indicates the uniform character of the original material

used. The period of treatment was purposely prolonged and the storage temperature held low, with the hope that any changes occurring as a result of the ethylene or other treatments might be cumulative, and hence less transitory and more easily detected and measured.

The values for the various carbohydrate groups in table II lend scant support to the hypothesis that ethylene treatment increases the sugar content of stored apples. The variations are no greater than might be expected from uniformly treated lots.

With ethylene treatment, the sucrose of Stayman Winesap was slightly increased but it was reduced in like amount in Ben Davis and essentially unchanged in Rome Beauty. Conversely, reducing sugars were slightly increased in Ben Davis but practically unchanged in Stayman Winesap and Rome Beauty.

The ethylene-chlorohydrin treatment appears to have reduced moderately the sucrose content in Stayman Winesap and Ben Davis, and slightly increased their reducing sugars, but the change is not pronounced and does not occur at all in Rome Beauty.

The starch content is low in all three varieties but is no less in the ethylene and ethylene-chlorohydrin treated fruits. Clearly neither ethylene nor ethylene chlorohydrin has reduced the starch content, the effect, if any, being in the opposite direction.

The acidity of the expressed juice indicates that again there is nothing to show that ethylene has affected the pH value of the apples. This measure also runs uniform. Since the apples were well matured when picked, this feature might not be expected to change materially, except perhaps to increase in pH value somewhat as softening proceeded.

The outcome of these experiments has resulted in failure to increase either the sugar content or the pH value of the juices of apples held in storage. Ethylene treatment did hasten the change of color from green to yellow, however, and also the rate of softening of the apple tissues. Other experiments with ethylene treatment at higher temperatures, not otherwise described in this report, gave similar results with respect to color change and softening but in a much shorter period of time.

Summary

Ethylene, ethylene chlorohydrin, and ultraviolet irradiation treatments of stored apples of Rome Beauty, Stayman Winesap, and Ben Davis varieties did not materially affect the chemical composition of the fruit or the pH value of the expressed juices as compared with similarly stored, untreated fruits. Ethylene treatment hastened the color change from green to yellow and likewise accelerated the softening of the apple tissues.

DEPARTMENT OF AGRICULTURAL CHEMISTRY
WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION
MORGANTOWN, W. VA.

LITERATURE CITED

1. CHACE, E. M., and CHURCH, C. G. Effect of ethylene on the composition and color of fruits. Ind. & Engr. Chem. **19**: 1135–1139. 1927.
2. ————————, and DENNY, F. E. Use of ethylene in the coloring of citrus fruit. Ind. & Eng. Chem. **16**: 339–340. 1924.
3. ENGLIS, D. T., and ZANNIS, C. D. The effect of ethylene upon the activity of diastase and invertase. Jour. Amer. Chem. Soc. **52**: 797–802. 1930.
4. HARVEY, R. S. Artificial ripening of fruits and vegetables. Minnesota Agr. Exp. Sta. Bull. 247. 1928.
5. HIBBARD, R. P. The physiological effect of ethylene gas upon celery, tomatoes and certain fruits. Michigan Agr. Exp. Sta. Tech. Bull. 104. 1930.
6. KOHMAN, E. F. Ethylene treatment of tomatoes. Ind. & Eng. Chem. **23**: 1112–1113. 1931.
7. METHODS OF ANALYSIS OF ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 2d ed. 1925.
8. RAMSAY, A. A., and MUSSO, L. A. Coloring of oranges with ethylene. Agr. Gaz. New South Wales **41**: 382–383. 1930.
9. REGEIMBAL, L. O., and HARVEY, R. B. The effect of ethylene on the enzymes of pineapples. Jour. Amer. Chem. Soc. **49**: 1117–1118. 1927.
10. ROSA, J. T. Ripening of tomatoes. Proc. Amer. Soc. Hort. Sci. Ann. Rept. **22**: 315–322. 1925.
11. ————————. Changes in composition during ripening and storage of melons. Hilgardia **3**: 421–443. 1928.
12. WOLFE, H. S. Effect of ethylene on the ripening of bananas. Bot. Gaz. **92**: 337–336. 1931.

LONG AND SHORT WAVE-LENGTH LIMITS OF PHOTOSYNTHESIS[1]

G. RICHARD BURNS

(WITH TWO FIGURES)

A complete description of the apparatus and the experimental procedure employed in these experiments has been given elsewhere (1). Briefly, it consisted of exposing the plant to one portion of the spectrum for two hours, determining the amount of carbon dioxide used, and then exposing it to such an intensity of a second portion of the spectrum that the amount of carbon dioxide used was the same as in the first case. Under this condition it was assumed that the same amount of photosynthesis had occurred in the two cases, and that the relative efficiency of the two radiations in causing photosynthesis was inversely proportional to their intensities. In the experiments described in this paper, the method employed to determine the limits of photosynthesis was to compare the amount of photosynthesis caused by radiation—well within the limits—with that caused by radiation about half of which was within the limit and half just outside. For example, the short wave limit was obtained by comparing the efficiency of radiation 5 (fig. 1) with radiation 4, and the long wave limit by comparing 5 with 2. The different portions of the spectrum were obtained by providing each of the light sources with a double filter cell, one compartment being 1 inch thick and the other 0.5 inch thick. The solution in the 1-inch cell was circulated and cooled at the rate of 5 cubic feet per hour. Table I gives the filters used, table II and figure 1 the transmission spectra of these filters. The wave-lengths given are the wave-lengths at the center of the thermopile at each measurement, the spectrum having been shifted the width of the thermopile between each measurement. Thus in table II the

TABLE I

FILTERS

COMPOSITION OF SOLUTIONS

No.	1-INCH CELL	0.5 INCH CELL
1	Water	Water
2	Water	50 gm. $K_2Cr_2O_7$ in 1 liter water
3	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	Water
4	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	500 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water
5	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	50 gm. $K_2Cr_2O_7$ in 1 liter water

[1] Contribution from the Vermont Agricultural Experiment Station, published with the consent of the Director.

eighth line, beginning 6461, means that between about 6696 and 6272 Å., filter 1 transmitts 105.3 parts of energy; filter 2, 102.0 parts; filter 3, 1.9 parts; etc.

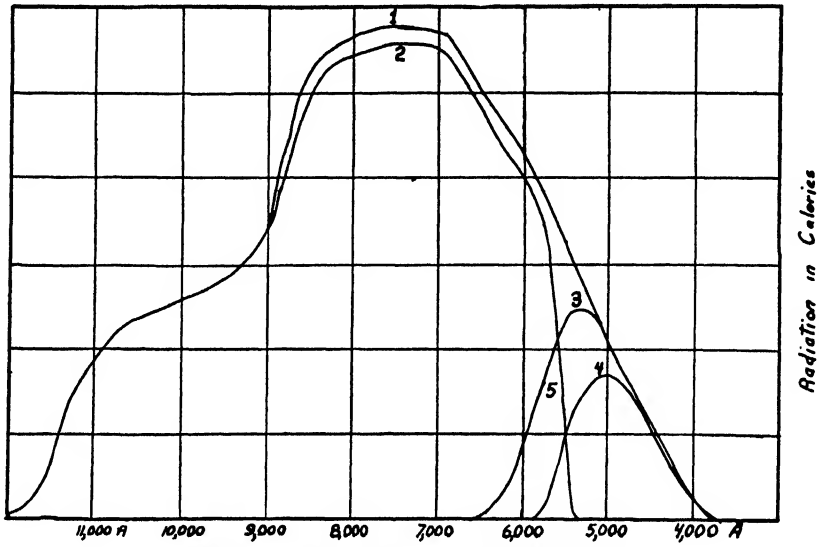


FIG. 1. Transmission spectra of filters.

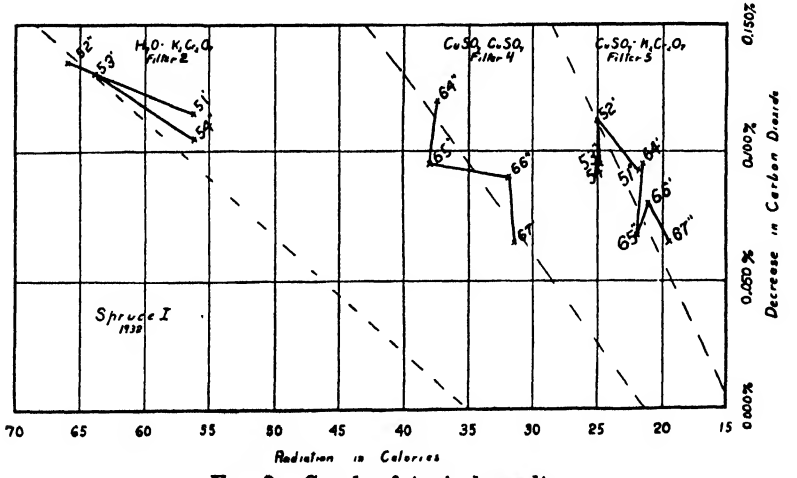


FIG. 2. Graph of typical results.

In table III the run number is the serial number of the day on which the experiment was performed (July 1 being 1) and the number of primes indicates whether it was the first or second determination on the tree that day. The second column gives the number of the tree; P1 for white pine number

TABLE II

TRANSMISSION OF FILTERS

WAVE LENGTH	FILTER 1	FILTER 2	FILTER 3	FILTER 4	FILTER 5
Å	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
14270	0.4	0.2			
12230	6.4	6.4			
10610	116.0	116.0			
9380	162.5	162.0			
8280	249.0	242.0			
7519	194.0	188.0			
6931	150.0	146.0			
6461	105.3	102.0	1.9		1.3
6082	76.3	73.5	12.5	0.2	12.1
5769	56.0	50.4	25.4	3.0	24.2
5502	40.9	16.6	28.2	10.7	12.9
5270	29.7	0.5	24.4	16.2	0.2
5071	21.6		19.6	16.2	
4898	15.8		14.8	13.6	
4743	11.8		11.3	10.7	
4602	9.0		8.4	8.1	
4480	6.6		5.6	5.8	
4368	4.7		4.0	4.2	
4267	3 4		2.9	2.9	
4173	2.5		2.0	2.0	
4088	1.6		1.3	1.4	
4010	1.1		1.1	1.1	
3936	0.7		0.4	0.4	
3867	0.4		0.2	0.2	
3803	0.1		0.1	0.1	

1, S1 for Norway spruce number 1, etc. The next column gives the filter used; the next two the decrease in carbon dioxide concentration expressed as percentage total gas (duplicate determinations). The column headed radiation is the radiation in calories per cm.^{2} per run $\times$ 10. These values are not corrected for the transmission of the bell-jars, about 89 per cent. The corrected radiation is the radiation that would be necessary to obtain exactly the same carbon dioxide decrease as in the companion run, and was determined from a graph of the values (fig. 2). The next column gives the percentage efficiency of one of the portions of the spectrum as compared with the other, and the final column shows the experimental error which would result from an error of 0.005 per cent. in the gas analysis and the maximum probable error in the graphical determination of the corrected radiation value.

In considering table III, it is interesting to note the change in efficiency exhibited by a tree toward a given portion of the spectrum on successive days. While the change is usually less than 10 per cent., it may be as high as 50 per cent. for the first two or three days of exposure to a new radiation:

TABLE III

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiation	Corrected radiation	Efficiency	Experimental error
					Cal. × *10*	*Cal.* × *10*	%	%
7′	P1	5	0.120	0.120	32.9			
7″	P1	3	0.135	0.135	32.5	31.2	106.0	3
8′	P1	3	0.180	0.175	40.6	43.3	86	4
8″	P1	5	0.200	0.190	37.4			
9′	P1	5	0.285	0.290	46.2			
9″	P1	3	0.235	0.240	49.2	56.4	82	4
11′	P1	5	0.345	0.340	50.5			
11″	P1	3	0.290	0.280	53.0	60.5	83	4
12′	P1	3	0.260	0.250	48.4	50.0	75	2
12″	P1	5	0.270	0.260	37.7			
14′	P1	5	0.365	0.365	50.9			
14″	P1	3	0.250	0.255	51.7	69.5	73	5
7′	P2	5	0.045	0.045	31.8			
7″	P2	3	0.050	0.050	29.3	28.4	112	3
8′	P2	3	0.090	0.085	36.0	53.5	79	8
8″	P2	5	0.185	0.185	42.5			
9′	P2	5	0.200	0.210	47.3			
9″	P2	3	0.185	0.190	50.9	54.3	87	3
11′	P2	5	0.280	0.270	56.2			
11″	P2	3	0.240	0.245	55.5	60.5	93	3
12′	P2	3	0.180	0.180	44.1	49.0	90	4
12″	P2	5	0.210	0.215	42.4			
14′	P2	5	0.290	0.290	49.8			
14″	P2	3	0.215	0.220	53.1	64.2	78	5
16′	P3	5	0.215	0.215	39.4			
16″	P3	3	0.240	0.240	44.8	41.4	95	4
18′	P3	3	0.145	0.145	30.0	27.0	79	7
18″	P3	5	0.120	0.125	21.3			
19′	P3	5	0.240	0.245	31.9			
19″	P3	3	0.245	0.245	38.0	37.7	85	2
20′	P3	3	0.170	0.170	33.3	31.5	82	4
20″	P3	5	0.155	0.155	25.8			
21′	P3	5	0.200	0.200	29.7			
21″	P3	3	0.205	0.210	32.2	31.3	93	5
22′	P3	3	0.170	0.170	30.5	34.8	75	6
22″	P3	5	0.210	0.210	26.0			
16′	P4	5	0.195	0.200	37.7			
16″	P4	3	0.205	0.200	34.5	34.2	110	10
18′	P4	3	0.135	0.140	28.4	25.0	90	5
18″	P4	5	0.105	0.110	22.4			
19′	P4	5	0.230	0.225	31.4			
19″	P4	3	0.205	0.210	34.6	36.7	86	3
20′	P4	3	0.190	0.190	32.4	31.5	93	3
20″	P4	5	0.180	0.180	29.4			
21′	P4	5	0.245	0.250	34.2			
21″	P4	3	0.190	0.190	34.2	40.8	84	4
22′	P4	3	0.165	0.165	30.4	33.7	81	5
22″	P4	5	0.200	0.195	27.4			
23′	P1	3	0.160	0.160	38.1	33.8		
23″	P1	4	0.135	0.135	40.3		84	6
27′	P1	4	0.190	0.195	45.2		85	8
27″	P1	3	0.280	0.280	49.1	38.4		
29′	P1	3	0.175	0.175	33.5	33.3		

TABLE III—(*Continued*)

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiations	Corrected radiation	Efficiency	Experimental error
					Cal. × 10	*Cal.* × 10	%	%
29″	P1	4	0.170	0.175	39.9		83	3
30′	P1	4	0.305	0.305	69.2		79	2
30″	P1	3	0.295	0.295	53.2	54.6		
31′	P1	4	0.105	0.115	33.7		85	3
31″	P1	3	0.120	0.115	29.7	28.8		
32′	P1	3	0.210	0.210	43.3	40.0		
32″	P1	4	0.185	0.185	50.3		80	3
23′	P2	3	0.125	0.125	40.1	40.5		
23″	P2	4	0.125	0.130	44.3		91	2
27′	P2	4	0.195	0.195	49.2		88	7
27″	P2	3	0.225	0.230	49.5	43.6		
29′	P2	3	0.175	0.170	34.8	30.3		
29″	P2	4	0.140	0.135	41.8		72	6
30′	P2	4	0.280	0.275	70.6		85	4
30″	P2	3	0.250	0.250	55.8	60.0		
31′	P2	4	0.105	0.105	36.7		80	6
31″	P2	3	0.125	0.120	31.9	29.5		
32′	P2	3	0.135	0.135	42.1	51.3		
32″	P2	4	0.190	0.185	51.8		99	8
38′	P3	3	0.140	0.140	16.9	18.7		
38″	P3	1	0.165	0.155	65.2		28.7	8
39′	P3	1	0.295	0.295	107.2		44.4	14
39″	P3	3	0.145	0.150	29.8	47.6		
40′	P3	3	0.210	0.210	41.8	47.0		
40″	P3	1	0.250	0.245	91.3		51.5	4
41′	P3	1	0.170	0.175	72.7		45.9	2
41″	P3	3	0.175	0.175	33.6	33.3		
42′	P3	3	0.180	0.180	35.3	34.4		
42″	P3	1	0.170	0.175	65.7		52.3	2
43′	P3	1	0.250	0.250	84.9		55.0	4
43″	P3	3	0.220	0.220	42.4	46.6		
44′	P3	3	0.225	0.220	41.8	36.8		
44″	P3	1	0.185	0.185	75.2		49.0	2
38′	P4	3	0.130	0.125	16.0	17.3		
38″	P4	1	0.140	0.145	64.6		26.8	6
39′	P4	1	0.280	0.280	109.5		49.6	10
39″	P4	3	0.140	0.140	33.3	54.3		
40′	P4	3	0.235	0.230	42.9	45.5		
40″	P4	1	0.250	0.250	92.8		49.0	3
41′	P4	1	0.170	0.170	73.6		44.0	3
41″	P4	3	0.175	0.175	33.1	32.4		
42′	P4	3	0.175	0.175	33.3	28.5		
42″	P4	1	0.140	0.135	61.0		46.7	5
43′	P4	1	0.215	0.215	85.3		50.5	2
43″	P4	3	0.210	0.205	42.0	43.0		
44′	P4	3	0.200	0.205	42.8	39.0		
44″	P4	1	0.175	0.175	76.9		50.7	5
49′	S1	2						
49″	S1	5						
50′	S1	5	0.005	0.005	12.3	21.7		
50″	S1	2	0.110	0.115	53.6		40.5	16
51′	S1	2	0.115	0.115	57.2		41.6	5
51″	S1	5	0.095	0.090	21.9	23.8		
52′	S1	5	0.115	0.115	24.9	26.8		

TABLE III—(*Continued*)

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiations	Corrected radiation	Efficiency	Experimental error
					Cal. × *10*	*Cal.* × *10*	%	%
52″	S1	2	0.135	0.135	66.0		40.6	4
53′	S1	2	0.130	0.130	63.9		45.1	6
53″	S1	5	0.095	0.095	24.8	28.8		
54′	S1	5	0.095	0.095	24.8	26.0		
54″	S1	2	0.105	0.105	56.2		46.3	4
49′	S2	2						
49″	S2	5						
50′	S2	5	0.050	0.050	11.6	17.7		
50″	S2	2	0.160	0.155	54.6		32.7	25
51′	S2	2	0.060	0.060	58.7		36.0	3
51″	S2	5	0.050	0.050	20.1	21.1		
52′	S2	5	0.060	0.060	24.0	27.0		
52″	S2	2	0.090	0.090	62.7		43.0	4
53′	S2	2	0.110	0.110	62.5		41.6	4
53″	S2	5	0.085	0.085	23.9	26.0		
54′	S2	5	0.100	0.100	24.3	24.0		
54″	S2	2	0.095	0.100	56.2		41.7	2
55′	P3	2	0.075	0.075	54.6		41.7	5
55″	P3	5	0.070	0.070	22.1	22.8		
56′	P3	5	0.085	0.085	22.3	18.6		
56″	P3	2	0.055	0.055	44.9		41.4	10
57′	P3	2	0.110	0.110	57.1		39.0	4
57″	P3	5	0.105	0.105	21.8	22.3		
58′	P3	5	0.120	0.120	21.7	21.7		
58″	P3	2	0.120	0.120	57.7		37.6	3
60′	P3	2	0.160	0.160	55.0		39.1	3
60″	P3	5	0.160	0.160	21.5	21.5		
55′	P4	2	0.110	0.115	56.0		47.7	6
55″	P4	5	0.080	0.085	23.3	26.7		
56′	P4	5	0.105	0.105	23.5	18.3		
56″	P4	2	0.055	0.055	45.5		40.2	11
57′	P4	2	0.110	0.105	61.7		38.9	3
57″	P4	5	0.100	0.105	23.5	24.0		
58′	P4	5	0.130	0.130	23.0	22.6		
58″	P4	2	0.125	0.125	61.3		36.9	3
60′	P4	2	0.175	0.175	60.6		44.0	5
60″	P4	5	0.140	0.140	23.4	26.7		
64′	S1	5	0.095		22.6	24.6		
64″	S1	4	0.120		37.4		65.8	4
65′	S1	4	0.095	0.095	38.0		64.5	4
65″	S1	5	0.070	0.065	22.0	24.5		
66′	S1	5	0.080	0.080	21.2	21.9		
66″	S1	4	0.090	0.090	31.9		68.7	3
67′	S1	4	0.065	0.065	31.4		62.5	2
67″	S1	5	0.065	0.065	19.6	19.6		
64′	S2	5	0.105		23.4	22.6		
64″	S2	4	0.095		38.0		59.5	3
65′	S2	4	0.125	0.125	39.2		70.5	8
65″	S2	5	0.070	0.075	22.4	27.6		
66′	S2	5	0.070	0.070	21.9	22.6		
66″	S2	4	0.080	0.075	33.6		67.3	3
67′	S2	4	0.035	0.035	32.4		63.6	4
67″	S2	5	0.025	0.025	20.6	21.6		

Run 39, P3, filter 3, −41%; filter 1, − 6%
Run 39, P4, filter 3, −50%; filter 1, −11%
Run 51, S1, filter 2, − 6%; filter 5, −13%
Run 51, S2, filter 2, −34%; filter 5, −42%

From the values in table III the weighted averages of table IV were obtained. The limits of photosynthesis were calculated by means of the transmission spectra of the filters and the weighted averages on the following assumptions:

1. The absorption and reflection of the plant does not change with changing wave-length. Since absorption is less and reflection greater in the center of the visible spectrum, this assumption would make the calculated limits somewhat too far apart.

2. The amount of photosynthesis is proportional to the number of quanta and not to the energy of the radiation. If the amount of photosynthesis were proportional to the energy, the red limit would be moved out about 160 Å. and the blue in about 120 Å.

TABLE IV

EFFICIENCY OF FILTERS AND LIMITS OF PHOTOSYNTHESIS

TREE	FILTERS		EFFICIENCY A IN TERMS OF B	LIMIT OF PHOTOSYNTHESIS
	A	B		
			%	Å
S1	2	5	42.8	7430
S2	2	5	40.5	7380
P3	2	5	39.7	7340
P4	2	5	41.5	7390
S1	4	5	65.4	4660
S2	4	5	65.3	4660
P1	4	5	66.1*	4650
P2	4	5	73.3*	4490
P1	3	5	80.0	4630
P2	3	5	85.4	4430
P3	3	5	82.8	4530
P4	3	5	86.0	4390
P1	4	3	82.6	4460
P2	4	3	85.8	4370
P3	1	3	49.8	7160‡
P4	1	3	48.3	7140‡
P3	1	5	41.2†	7220‡
P4	1	5	41.5†	7230‡

* Calculated from 3–5 and 4–3.
† Calculated from 1–3 and 3–5.
‡ Violet limit set at 4540.

3. The quantum yield does not change with changing wave-length.
4. The limit of photosynthesis is sharp.

These assumptions are being made the subject of further study, as is the ability of the plant to adapt itself to the use of different wave-lengths. In calculating the limits, the transmission spectra were put on an approximate quantum basis by multiplying the energy at a particular spectrograph setting by the wave-length at the center of the thermopile. The radiation intensities were multiplied by the average quanta per calorie value of the particular filter as determined from the transmission spectra.

The 2–5 and the 4–5 filter combinations (table IV) should give the most accurate results and the other values are included for comparison.

It should be noticed that P1 had the lowest value in the 3–5 runs and doubtlessly the limit obtained from P2 is more typical. An error of 10 per cent. in the efficiency would shift the values 100 Å in the red and 140 Å in the blue. On the other hand the spectrograph readings, the calculations and the filters employed should yield more accurate results in the blue. In considering the blue limit it should be remembered that WARBURG and NEGELEIN (**2**), with algae, found only a 25 per cent. decrease in the quantum yield at 4360 Å. This difference in the short wave limit is probably due to the characteristics of the plants or to the fact that the trees were purposely not acclimated to blue and violet light.

Summary

A study of the limits of photosynthesis of white pine and Norway spruce showed that these trees were able to use all of the visible spectrum with the exception of part of the blue and all of the violet.

VERMONT AGRICULTURAL EXPERIMENT STATION
BURLINGTON, VERMONT

LITERATURE CITED

1. BURNS, G. R. Photosynthesis in various portions of the spectrum. Plant Physiol. **8**: 247–263. 1933.
2. WARBURG, O., and NEGELEIN, E. Über den Einfluss der Wellenlänge auf den Energieumsatz bei der Kohlensäureassimilation. Zeitschr. physik. Chem. **106**: 191–219. 1923.

EVAPORATION IN ITS THERMODYNAMIC RELATION TO THE COHESION THEORY AND TO IMBIBITIONAL AND OSMOTIC PRESSURES OF CELL CONSTITUENTS

CLYDE HOMAN, T. F. YOUNG, AND CHARLES A. SHULL

(WITH THREE FIGURES)

That the upward flow of dilute solutions in the tracheae of the taller plants results from a tension in the column of liquid sustained by its cohesive forces (correlated with evaporation from the cells at the top of the column) has been postulated in the well known cohesion theory of DIXON and JOLY (3). The purposes of this paper are to show that the maximum tension or negative pressure in the tracheae depends on the relative humidity of the atmosphere, to show how evaporation operates to produce this tension, and to call attention to a thermodynamic equation by which possible and theoretical values may be calculated. The use of a similar equation in determining osmotic and imbibitional pressures of cell constituents and soils is also illustrated.

A simple mechanical apparatus for the production and demonstration of negative pressures produced by evaporation is shown in figure 1. The

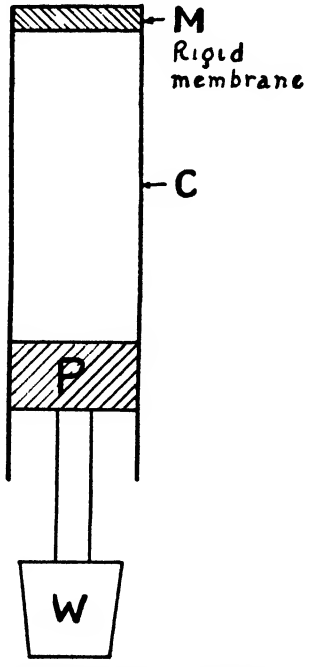


FIG. 1. Apparatus for production and demonstration of negative pressures by evaporation.

membrane, M, which is sealed to the top of the cylinder, C, contains pores which permit water to pass from the cylinder and evaporate into the atmosphere. The cylinder is filled with gas-free water. The piston, P, is free to move, but is so made that no air may leak past it. We shall neglect its own mass and that of the liquid in the cylinder, and concentrate our attention on the variable mass, W.

When water evaporates from the upper surface of the membrane, the volume of the liquid in the chamber decreases; and the piston, if free, is drawn upward. Now as we observe the rise of the piston we may increase the weight, W, until the resulting downward force has produced such a negative pressure or tension in the liquid that further loss of water by evaporation (and consequent rise of the piston) is stopped. This force divided by the area of the piston (less the atmospheric pressure acting on P) is, of course, the negative pressure within the cylinder. Should W be further increased, water vapor would condense from the atmosphere, pass through the membrane into the cylinder, and the piston would descend. The column of water in the cylinder corresponds to that in the tracheae, and the membrane corresponds to the mesophyll cells. For each set of conditions (temperature, relative humidity) there will be a unique value of the negative pressure which can be calculated by an equation first used by POYNTING (5):

$$\frac{d P_l}{d P_v} = \frac{V_v}{V_l} \tag{1}$$

in which P_l is the pressure within the liquid, P_v is the (partial) pressure of vapor above the membrane, and V_l and V_v are the molal volumes (the volume of one mole of liquid or vapor) of the liquid and gaseous water respectively.

The molal volume of the vapor may be represented approximately by: $V_v = \frac{RT}{P_v}$ in which R is the gas constant, 82.06 cc. atm. mole^{-1} deg,$^{-1}$, and T is the absolute temperature. The volume of the liquid does not vary greatly for moderate changes in pressure. If we represent it simply by a constant, V_l, equation (1) may be integrated:

$$P_l - P_l' = \Delta P_l = \frac{RT}{V_l} \ln \frac{P_v}{P_v'} = 2.3026 \frac{RT}{V_l} \log \frac{P_v}{P_v'} \tag{2}$$

Here P_v is the partial pressure of water vapor above the membrane when equilibrium is established, P_v' is the vapor pressure of the water when the pressure on the liquid is one atmosphere (1.013×10^6 dyne cm.$^{-2}$). [This is nearly the vapor pressure (within 0.1 per cent.) of H_2O when the liquid water is subjected to its own vapor pressure]. P_l' is the atmospheric pres-

sure to which P_v' corresponds, *i.e.*, one atmosphere, and P_l is the variable pressure within the liquid to which P_v corresponds. When P_v/P_v' is less than one, $P_l - P_l'$ is negative. When P_v/P_v' becomes sufficiently small, P_l itself becomes negative.

The pressure difference inducing the flow of sap within the plant is the pressure on the cells of the roots minus the pressure produced in the mesophyll sap by evaporation, *i.e.*, $P_l' - P_l$. This difference (which is one atmosphere minus the pressure, P_l) we shall designate for convenience the *stromogenic pressure difference*. When the stromogenic pressure difference is greater than one atmosphere, a negative pressure or tension exists in the liquid, and in such cases the term stromogenic *tension* may be employed.

In table I, column 2, are listed *calculated values* of the maximum stromogenic tension which can be produced in pure water by evaporation into an atmosphere in which the relative humidity has the values listed in column 1. In making these calculations we have neglected the change (with pressure) of the molal volume of water.

Extrapolating the density data of BRIDGMAN (1), we have also obtained the values in column 3. The corrections, involving extrapolation, may not be precise but serve to illustrate the magnitude of the errors in column 2. There are two minor approximations in the calculation. We have used the perfect gas law, and Dalton's law of partial pressure. The data given are, of course, for pure water, and not for dilute solutions, such as the soil solu-

TABLE I

MAXIMUM STROMOGENIC PRESSURES AT 20° C.* FOR VARIOUS RELATIVE HUMIDITIES

RELATIVE HUMIDITY	STROMOGENIC TENSION	
	CALCULATED, NEGLECTING VARIATIONS IN V_l	CORRECTED FOR VARIATIONS IN V_l
%	*atm.*	*atm.*
99.9	1.33	1.33
99.5	6.68	6.68
99.0	13.40	13.39
98.0	26.93	26.91
95.0	68.4	68.3
90.0	140.4	140.0
70.0	475	471
50.0	924	906
30.0	1605	1550
10.0	3069	2880

* According to the equation, the usual variations in temperature would affect the pressures but little, as these are proportional to the absolute temperature.

tion and sap in the tracheae. For such dilute solutions appropriate corrections could be applied, for the vapor pressure of water from the solution, and for the difference in the molal volume (partial molal volume—see later) of water in the solution.

It is not likely that (negative) pressures within liquid water can ever reach the higher values indicated in the table. The greatest tension obtained experimentally in water by DIXON (2) was 158.4 atmospheres, and in plant sap 207 atmospheres. Higher values, however, may be possible, and theory indicates that they are.

Osmotic and imbibitional pressures

Figure 2 represents an idealized apparatus for illustrating two convenient methods for applying and measuring osmotic and imbibitional pres-

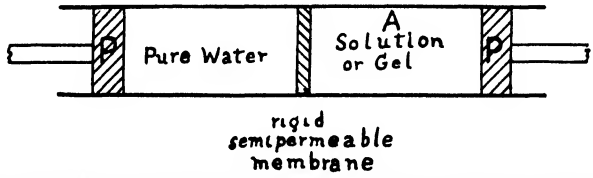


FIG. 2. Apparatus for applying and measuring osmotic and imbibitional pressures.

sures. The first, the usual method, is to maintain the pressure on the pure liquid constant, and to apply and measure the pressure which must be applied to A to prevent osmosis through the semipermeable membrane at the center. The second method is to maintain the solution or gel at a constant pressure, and decrease the pressure on the pure liquid B (to negative values if necessary) until osmosis or imbibition is prevented. The two values so obtained are not in general exactly the same, since the effect of a decrease in pressure on the molal volume is not the same as an increase, and because the molal volume of the pure liquid is not the same as the partial molal volume[1] of the solvent in the solution. The differences are small for dilute solutions, and we shall refer later to the values determined by the second method, *i.e.*, that involving a reduction of the pressure on the pure solvent.

The water in every solution or gel having a given osmotic or imbibitional pressure, Π, would be in equilibrium with an atmosphere of some definite

[1] The partial molal volume, $\overline{v}$, as defined by G. N. LEWIS (4) is the increase in volume per mole of water (or any other substance) added when the amount added is infinitesimal, *i.e.*, $\overline{v} = dV/dn$ where V is the volume of the solution containing n moles of water. $\overline{v}$ is not difficult to determine experimentally. Like the molal volume of the pure liquid, it is a function of pressure and temperature. It is also a function of the composition of the solution, and differs very little from the molal volume of the pure liquid when the solution is dilute.

relative humidity which may be calculated. If Π represents the osmotic pressure of the solution as measured by method 2, then the water in the solution is in equilibrium with water under the negative pressure Π. The partial pressure of water vapor in equilibrium with the latter can be calculated from equation (2). Since the solution must be in equilibrium with water vapor at the same partial pressure, it follows that the osmotic pressure Π of any solution is related approximately to the relative humidity of the atmosphere in equilibrium with it, by the equation:

$$\Pi = -\frac{RT}{V_1} \ln \frac{P_v}{P_v'} \tag{3}$$

This is a well known thermodynamic equation.

Consider for example a solution, the osmotic pressure of which is 68.3 atmospheres. Such a solution placed in the right chamber of figure 2 would of course be in equilibrium with water in the left chamber under a tension of 68.3 atmospheres. The latter would be in equilibrium with the atmosphere when the relative humidity is 95 per cent. (see table I), and the solution is in equilibrium with the same atmosphere.

If a negative pressure is applied to a solution the osmotic pressure of which is not negligible, the relative humidity corresponding may be calculated approximately (accurately for dilute solutions at low pressures) by use of equations (2) and (3) together. Suppose the solution whose osmotic pressure is 68.3 atmospheres is placed in the chamber of figure 1. If the relative humidity is reduced from 95 to 90 per cent., to prevent evaporation the weight, W, would have to be such that a tension of approximately 140 – 68.3, or 71.7 atmospheres were produced in the cylinder. If no pressure were applied, evaporation would go on until the concentration of the solution corresponded to an osmotic pressure of 140 atmospheres. If the relative humidity were raised from 95 to 99 per cent., a pressure of $68.3 - 13.4 = 55$ atmospheres would have to be exerted by the piston P. In the absence of any such pressure, water would be absorbed from the atmosphere, and the solution would become more dilute until its osmotic pressure had been reduced to 13.4 atmosphere.

Figure 3 will be used to illustrate some of the points brought out previously in connection with mesophyll cells abutting on tracheae. A is a membrane, as in fig. 1, but it is not rigid and is free to move up and down the cylinder. B is a semipermeable membrane held rigid, while P is a piston as in fig. 1. If compartment AB contains a solution whose osmotic pressure is 68.3 atmospheres, if the relative humidity above the membrane A is 95 per cent. (see table I), and if a pull equivalent to 68.3 atmospheres is exerted by W on piston P, the system will remain stationary. Should the relative humidity drop to say 90 per cent., however, membrane A will move

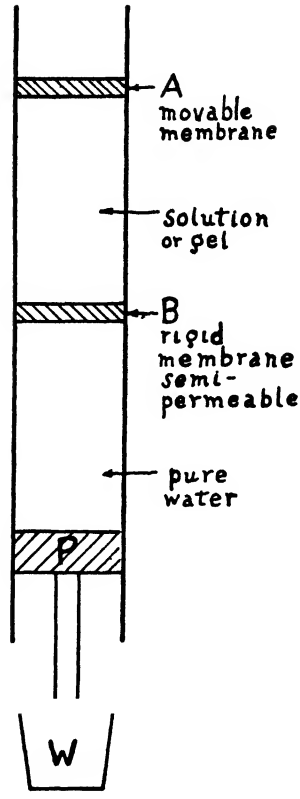


FIG. 3. Apparatus model to illustrate the action of mesophyll cells.

downward as the concentration and the osmotic pressure increase. The piston P will then move upward as water diffuses through membrane B and evaporates from membrane A. If W, however, is increased at the time the relative humidity is decreased to 90 per cent., until a pull of 140 atmospheres is exerted, water will pass downward through B and evaporation will take place from A (which would move downward) until the osmotic pressure becomes 140 atmospheres due to increasing concentration. Equilibrium would then be reached. Thus it is that were it not for delay in reaching equilibrium resulting from slowness of diffusion, the stromogenic tension and the osmotic pressure of the cell vacuoles would be constantly the same, and in equilibrium with each other.

Since diffusion through cell walls and in and out of submicroscopic pores in the walls and through stomatal pores must be a very slow process, whereas the flow of sap may be relatively rapid, it is probable that, under actual conditions, nowhere near the theoretical maximum pressures are ever produced in active tissues. In addition, external and internal conditions are

constantly changing, and calculations made from the relative humidity of the external air could not be taken as a basis for calculating actual stromogenic tensions, etc., but would indicate only the possible tensions that could be produced, were all conditions suitable. They do show, however, the high reserve in lifting ability over what even the tallest trees require. Even in low-growing plants a considerable portion of this reserve lifting ability may be utilized at times to overcome friction in the tracheae, etc., and to withdraw water from dry soils in which water is held at high imbibitional pressures.

Stromogenic tension at wilting point of leaves

Since the relative humidities of natural atmospheres are frequently as low as 20 to 30 per cent., it is evident that the construction of plants enables them to prevent the evaporation of water to a marked degree, or they would quickly perish. It is possible that extreme xerophytes (*Opuntia* spp., etc.), and possibly epiphytes may be the only types of plants that could continue to live and grow in an atmosphere containing water at very low partial pressure. Mesophytes would promptly wilt when the water supply is shut off at the roots and the internal atmosphere drops only a small amount below saturation. In such types 68 atmospheres would be a high average value for the osmotic pressure of the cell vacuoles, and wilting would occur at 20° C. when the internal atmosphere of the leaf reached a relative humidity of 95 per cent. (assuming that the osmotic pressure is 68 atmospheres). Since the relative humidity of the external atmosphere is much below 95 per cent., it is obvious that the epidermal cells with their cutinized surfaces and stomatal pores must operate efficiently in maintaining the humidity of the atmosphere surrounding the thin-walled mesophyll cells in a state of approximate saturation.

It would no doubt be possible to determine the relative humidity of the internal atmosphere of the leaf at the time of wilting, which would be closely related as described previously to the maximum possible stromogenic tension which these leaves could develop at wilting. The detached leaves could be put into a closed chamber and the point determined at which a decrease in the relative humidity produced an observable loss of turgor, while an increase in humidity restored the loss. (The definiteness of this point would vary with different leaves, and it is conceivable that certain types of leaves would not readily indicate the loss of turgor in the internal cells). The relative humidity could be found by analysis of a sample of the air from the chamber, or by calculation from the water content of sulphuric acid placed in the chamber. The use of a slightly pulsating pressure in the chamber might be desirable to hasten the approximation of equilibrium conditions.

Application to soils

Suppose a sample of soil were placed in a closed container and allowed to come to equilibrium with air of some selected relative humidity. If the relative humidity were then lowered, water would be lost by the soil. The loss, which could be determined by weight, would represent the amount of water held within the range between the two imbibitional pressures corresponding to the respective humidities. Thus if the soil sample lost a certain amount of weight while slowly changing from equilibrium conditions at 70 per cent. relative humidity to equilibrium conditions at 50 per cent. relative humidity, this loss would represent the amount of water absorbed with a pressure between 900 and 480 atmospheres.

Habitat measurements

By use of the methods here outlined one can calculate and compare the stromogenic tensions, and the imbibitional and osmotic pressures that could be developed in different environments, and at different times of day in the same environment. Thus in table II is shown the mean calculated stromogenic tensions for the months of January to July, 1932, at Washington, D. C.

The data are based on computations using Weather Bureau records[2] taken at 8:00 A.M., noon, and 8 P.M. Each value is based on the average relative humidity and temperature at the given hour for the entire month.

TABLE II

AVERAGE STROMOGENIC PRESSURES ATTAINABLE AT CERTAIN HOURS FOR THE FIRST SEVEN MONTHS OF 1932 AT WASHINGTON, D. C. DATA FROM WEATHER BUREAU

MONTH	8:00 A.M.	NOON	8:00 P.M.
	atm.	*atm.*	*atm.*
January	400	700	600
February	400	800	1000
March	500	900	800
April	600	1200	1000
May	500	1000	800
June	500	900	700
July	500	900	800

On the dryest day of January, 1932 (the 31st), these figures were, at 8:00 A.M., 1300 atm.; at noon, 1900; at 8:00 P.M., 1500.

Psychrometric readings taken among the dunes around Lake Michigan indicate occasionally relative humidities as low as 20 to 30 per cent., and an imbibitional pressure at such times equivalent to about 2000 atmospheres.

[2] The original meteorological data were furnished by Mr. M. C. BENNETT, Acting Chief of the Climatological Division of the Weather Bureau.

Laboratory air is usually arid, and seeds kept in laboratory storage will have a greater imbibitional pressure than seeds which are taken air dry from a natural environment. This is due to the greater evaporating tendency of water in laboratory air. The values found for seed colloids by SHULL (**6, 7**) using methods involving osmotic and vapor pressure equilibria, 950 to 1250 atmospheres, are therefore higher than the average values given in table II. The close agreement of the calculated imbibitional pressures required to prevent evaporation in natural atmospheres with the observed colloidal imbibitional pressures in arid dry seeds are in satisfactory agreement with the original measurements of these materials.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BRIDGMAN, P. W. The compressibilities of certain liquids. Int. Crit. Tables. Vol. III. P. 40. McGraw-Hill Book Co., 1928.
2. DIXON, H. H. Transpiration and the ascent of sap in plants. Chapter V. Macmillan Co. 1914.
3. ————, and JOLY, J. On the ascent of sap. Phil. Trans. Roy. Soc. London **186B**: 568——. 1895.
4. LEWIS, G. N. A new system of thermodynamic chemistry. Proc. Amer. Acad. Arts and Sci. **43**: 273——. 1907. (Consult LEWIS, G. N., and RANDALL, MERLE. Thermodynamics. p. 34. McGraw-Hill Book Co. New York. 1923).
5. POYNTING, J. H. Change of state. Solid-liquid. Phil. Mag. (5) **12**: 32–48. 1881.
6. SHULL, C. A. Semipermeability of seed coats. Bot. Gaz. **56**: 169–199. 1913.
7. ————. Measurement of the surface forces in soils. Bot. Gaz. **62**: 1–31. 1916.

PREPARATION OF HUMATE IRON AND OTHER HUMATE METALS

C. KENNETH HORNER, DEAN BURK, AND SAM R. HOOVER

Introduction

It has been shown that humate iron is a highly active stimulator of bacterial growth and an excellent source of iron for the general nutrition of plants, and that it is often more convenient to employ than other forms of iron now available, such as the sulphate, citrate, or tartrate (**1, 2, 3, 6**). It is, moreover, not precipitated in physiologically alkaline solutions nor by high concentrations of phosphate. Our previous studies in this connection were not concerned with efficient methods of preparation of humate iron, since most of the work involved microcultures of bacteria and hence relatively small requirements of material. In view of the considerable interest in this form of iron, and other humate metals, which appears to have developed among plant physiologists, the conditions essential to obtaining the large amounts needed in the case of higher green plants have been worked out and are reported herewith.

Synthetic humate

In the preparation of the metal-free synthetic humate itself from sugar and hot strong mineral acid, the optimum percentage yield is confined to somewhat narrow ranges of the several determining variables. To obtain three-fourths or more of the optimum percentage yield, the following requirements hold in general: sugar, 3 to 20 gm. per 100 cc. acid solution; acid, 25 to 40 cc. conc. H_2SO_4 per 100 cc. acid solution; temperature and duration of heating, 2 to 3 hours at the boiling point (115° C.). The percentage yield decreases markedly above a sugar concentration of 20 gm. per 100 cc. because of greatly enhanced formation of caramel and humus compounds insoluble in dilute alkali. The absolute yield may be increased twofold as the sugar concentration is increased from 10 gm. per 100 cc. up to saturation, but the yield of secondary products is increased three to sixfold. Glucose, starch, sucrose, molasses, cellulose (filter paper), and sawdust give yields equivalent within 25 per cent.; dextrin and xylose appear somewhat inferior. Factors of cost, availability, and purity would determine the substance to be used. The writers have used sucrose and glucose chiefly. HCl is only about one-fourth as effective as H_2SO_4, at the optimum concentrations, and has the disadvantage of being volatile. Glacial H_3PO_4 is considerably less effective than HCl. Heating on the steam bath at 80° C. requires 48 hours to produce the yield obtained in 2 hours by heating over a flame or in an autoclave at 115° C. It is preferable to

employ volumes of at least 100 cc. of acid-sugar digestion mixture in containers several times this capacity. The iron or metal salt is no longer added to this mixture, as formerly, but to the finally purified neutral potassium humate, the details of preparation of which now follow.

One liter of 30 volume per cent. H_2SO_4 (*i.e.*, containing 300 cc. conc. H_2SO_4) is brought to the boiling temperature, 100–150 gm. sugar added, and the mixture allowed to simmer for 3 hours. It is then cooled and centrifuged in non-corrosive vessels, discarding the liquid phase. Concentrated KOH (or NaOH) solution is added to the solid phase until upon being partially dissolved the solution becomes alkaline to phenolphthalein, the latter being employed as an outside indicator and used with somewhat diluted portions of test solution. The material is recentrifuged, using either an ordinary centrifuge or a Sharples supercentrifuge. The solid phase contains alkali-insoluble humus and is discarded after one or more additional washings with dilute alkali to recover small amounts of occluded humate. The alkaline extract and washings, containing relatively pure, soluble potassium humate, are combined and further purified by at least one more cycle of precipitating with H_2SO_4 at pH 3 to 4 (brom phenol blue as outside indicator), centrifuging, discarding the slightly acid liquid phase, and dissolving the solid hydrogen humate by neutralization with 1 to 5 per cent. KOH to give a neutral or slightly alkaline solution (brom thymol blue employed as outside indicator). The concentration of humate in the final stock solution thus prepared may be determined by precipitating the humate in a 1–5 cc. aliquot portion in a weighed centrifuge cup, centrifuging, discarding the liquid, drying at 100°C., and reweighing. Occluded white K_2SO_4 sometimes present in the dried humic acid may be corrected for by washing with water, recentrifuging, and redrying as before, or by combusting the dried sample in a crucible and determining the ash. A yield of 15 to 30 gm. humate (10 to 20 per cent.) should be obtained from an original 150 gm. sugar.

A microcombustion carried out by MILDRED S. SHERMAN on synthetic humic acid prepared from glucose by this improved method and washed as free from ash as possible gave the composition ratio: C, 64.95; H, 3.85; ash (chiefly K_2SO_4), 0.5; and O (by difference), 30.7. This corresponds on an ash-free basis to an atomic ratio $C_{5.44}H_{3.84}O_{1.92}$, an empirical formula $(C_6(H_2O)_{2.1})_n$, and a molecular weight of $(109.9)_n$, where n is not necessarily a whole number. The synthetic humic acid is thus chiefly a condensation product of sugar, with the loss of about two-thirds of the molecular water. Little or no oxidation is involved, unless of a Cannizzaro or similar internally compensating type. Methoxyl groups ($-OCH_3$) occur.

The theoretical yield of humic acid from glucose calculates out as (109.9/180.1)(100), or 61.0 per cent., and is considerably less than 100 per

cent. because of the much lower molecular weight of humic acid per unit of carbon. The experimental yield of 10 to 20 per cent. is thus about one-fourth the maximum theoretically possible. In view of the numerous side-reactions occurring, this yield, while conceivably still improvable, must be regarded as satisfactory, especially as it represents an improvement of 50 to 100-fold over the formerly obtained 0.2 per cent.

The preparation of synthetic humate from sugar has been studied to a certain extent by FRAPS (5). No separation was made of the large amount of charred carbon and other alkali-insoluble and acid-insoluble humus material from the acid-insoluble, alkali-soluble humic acid proper, and hence the results obtained were not comparable with those presented here. Much of the basic chemistry and methods of preparation of both the synthetic and natural humates was already well worked out one hundred years ago by SPRENGEL (7), but not from the detailed point of view of interest and applicability here presented.

Natural humate

Natural humate is extracted from soils as before (1, p. 416), except that only one or two cycles of purification are strictly necessary, instead of four or five as then recommended. Garden soils should yield 1 to 4 gm. humate per 100 gm. soil, and more in the case of peat. Any naturally decomposed organic matter may be employed. Natural humate differs from synthetic in consisting of a large number of organic compounds and inorganic elements, and also nitrogen. It is not yet known how many, if any, of the organic compounds are identical in both cases, but close relationships with respect to certain of the fundamental and important properties are definitely involved. The iron content of natural humate will vary from about 0.1 to 1.5 per cent. as the HCl-washing preliminary to the alkali extraction (1, p. 416) is varied from 0 to 24 hours; the iron is derived from the soil substances.

Humate iron and iron humate

In preparing humate iron, an inorganic iron salt is added directly to a neutral solution of potassium humate of known humate concentration. A concentrated solution such as 10 or 20 per cent. $Fe_2(SO_4)_3.9H_2O$ is convenient to employ. All preparations of natural or synthetic humates so far obtained have taken up at room temperatures at least 10 per cent. by weight of Fe, generally 15 per cent., and at times 20 to 25 per cent. The maximum percentage of iron held in solution by humate is substantially independent of the concentration of humate in the stock solution, at least over the 50-fold range of interest in connection with stock solutions, namely, 1 to 50 mg. per cc. It is generally desirable in the case of any particular humate to determine the maximum Fe/humate ratio in a small aliquot portion, and

then add iron to the main bulk of the stock solution to give a ratio 10–50 per cent. smaller.

Ten to 20 per cent. Fe in the soluble potassium-humate-iron preparation approximates the proportion of iron in the ordinary iron sulphate, tartrate, or citrate, but the manner of combination appears to be definitely different, hence *the term humate iron has been tentatively employed for iron thus held in solution, as compared with the compound iron humate highly insoluble in water.* Adsorption and colloidal states may be involved, but a basic substance is formed in the chemical sense that an amount of strong alkali approximately equivalent to complete neutralization of the potential acidity of the iron salt, with the formation of an alkali salt and the base ferric hydroxide, is necessary for the complete and stable solution of the iron in the neutral potassium humate solution. Humic acid itself, moreover, is not to be placed in the same category with simple organic acids.

The potential acidity of the iron salt added to the humate solution lowers the pH, and a precipitate of chiefly iron humate and some hydrogen humate is formed which is brought into solution again by further addition of strong alkali. The volume of solution is made up to some suitable value and the concentrations of humate and iron recalculated. Convenient concentrations for stock solutions are 2 mg. Fe and 15–25 mg. humate per cc., so that an initial dosage of 1 cc. per liter of nutrient medium provides 2 p.p.m. Fe, which, without further replenishment, should carry most bacterial cultures or green plants well toward maturity. Stock solutions will last indefinitely without further significant change. If desired, iron-hydrogen humate, obtained by precipitation of humate iron at pH 3 or less, centrifugation, and decantation, may be dried, so stored, and later dissolved with alkali as needed. If some chemical supply-house were to prepare this material and place it on the market for the benefit of investigators of nutritional problems, the solid form would probably be preferable, as in the case of many comparable indicators and dyes.

The formation of insoluble iron or hydrogen humate is not essential to the process of humate iron stabilization, as can be shown by making the potassium humate solution appropriately alkaline with KOH before the iron salt is added, so that upon mixing the two clear solutions the pH immediately assumes an equilibrium value between 7 and 9; no precipitate is observed to form unless momentarily due to insufficiently rapid mixing. Above pH 10 the maximum amount of iron kept in solution by a given amount of humate becomes somewhat reduced, the more so as the alkalinity is increased. In this same connection, a very great initial alkalinity of the humate solution will precipitate ferric hydroxide and greatly retard the rate and amount of formation of soluble humate iron.

A ferrous salt, if employed in place of a ferric salt in the preparation

of neutral humate iron, is oxidized completely to the ferric state by atmospheric oxygen in a few minutes, as we have found with the Warburg manometric apparatus. Hence there appears to be no advantage in employing ferrous rather than ferric iron. The ferrous salt does not reduce the humate appreciably during its oxidation, the rate and extent of which by oxygen gas are substantially the same in the presence and absence of humate, the pH being kept constant and either alkaline or very mildly acid.

Attention should be called to the fact that both natural and synthetic alkali humates are subject to precipitation by relatively high concentrations of one particular element found in many plant nutrient media, namely, calcium. Fortunately the lowest concentration of soluble calcium generally capable of causing significant precipitation, about 0.01 M, is far above that employed in bacterial media (0.001 M or less) and somewhat above that employed in the great majority of green plant nutrient media (0.002 to 0.005 M). All humates appear to be completely precipitated at 0.01 to 0.05 M Ca. The actual extent of precipitation depends chiefly upon the given concentration of calcium, but also somewhat upon the percentage of iron or metal attached to the humate, the particular humate preparation involved, and the concentration of humate. It has been observed empirically that the greater the capacity of a given humate to hold iron in solution (as humate iron) the greater the concentration of calcium required to cause humate precipitation (as calcium or iron humate). Certain plant nutrient media containing high concentrations of calcium may be found to precipitate some of the humate in particular humate preparations. In these cases it may be practicable, and not disadvantageous, to lower the calcium concentration to 0.003 M. It may also be desirable to decrease the ratio of iron to humate in the humate iron preparation. Magnesium precipitates humates only at concentrations five to ten times those required in the case of calcium. Precipitated calcium humate is readily soluble in alkali hydroxide so long as the concentration of free calcium ion is low, about 0.01–0.02 M. When for any reason the calcium ion concentration is considerably higher than this value, it may be reduced below it by addition of oxalate or some similar substance which precipitates calcium ion, and any precipitated calcium humate originally present will then go into solution upon addition of any necessary KOH. This circumstance is very fortunate in the process of extracting natural humate from highly calcareous soils, or from decomposed filter press cake, when for some reason these substances are first acidified and partially dissolved. The latter, a by-product of sugar manufacture, contains a high percentage of excellent natural humate, but also a high percentage of calcium, which with acidification would ordinarily prevent satisfactory solution of the humate in alkali, and would, in the absence of oxalate treatment, have first to be removed with great difficulty by means of extended acid washings.

Humate metals

Humate iron is only one member of a large class of similar humate metals. Al, Cu, Zn, Ni, Co, Hg, Ti, Cr, Mn, etc., all form hydroxides insoluble somewhere over the physiological pH range 3 to 9. Salts of these metals added in not too great excess lower the pH of a neutral potassium humate solution with the formation of more or less precipitated metal humate. These metal humates are dissolved upon further addition of alkali to give soluble, approximately neutral, humate metals. The maximum percentage of soluble metal taken up in solution by humate is about the same for each metal, 5 to 20 per cent. by weight of the humate. This maximum percentage is practically independent of the concentration of humate. It represents, moreover, the maximum for the total metal held in solution when several metals are added simultaneously, *i.e.*, the metals compete with one another for maintenance in solution. When metal is added just exceeding the maximum percentage, the insoluble hydroxide, and not the soluble basic humate, of the excess metal is formed upon making the pH neutral with alkali. But if the excess is several times greater than the amount held in solution, the whole of the humate is eventually precipitated in a form not easily dissolved even in strong alkali. The humate insolubility in this case depends markedly upon the initial humate concentration. In all these phenomena the various metals named behave like iron. It is obvious that in forming stock solutions care must be taken not to add too great an excess of metal, or some metal hydroxide or even metal humate will occur as a precipitate in the stock solution brought to neutrality. ERDENBRECHER (**4**) has described somewhat similar humate metal preparations.

A small class of metals, including Ca, Ba, Mg, etc., exists whose salts do not greatly alter the pH of a neutral potassium humate solution and whose hydroxides are relatively soluble over the entire physiological range. The ions of these metals form, when added to potassium humate solution in sufficient concentration, precipitated metal humates (*e.g.*, calcium humate, magnesium humate) which are also very difficultly soluble in strong alkali. The precipitating concentration depends here also markedly upon the concentration of humate as well as of metal. In a sense these metals may be considered as destroying the colloidal condition of the humate.

Natural humate, containing as it doubtless does traces of practically every element of significance in the growth of green plants—and in quite possibly highly available form—would appear to be a somewhat ideal substance to provide so-called "shot-gun" nutrition, especially when enriched with 5 to 15 per cent. iron and supplied as 10 to 20 parts per million of humate (or more), so as to provide iron at a concentration of a few parts per million and traces of the other elements at thousandths to tenths of a part per million. This iron-enriched natural humate could well be used in most culture work where the purpose is to obtain good healthy plant

growth in media varied in connections other than traces of elements. The use of the synthetic humate, which is somewhat more difficult and expensive to prepare, could be reserved for cases where it is desired to study the effect of intentionally added traces of specific elements and where application of the natural humate would obviously be undesirable because of the possibility of its containing these traces in significant amounts.

Summary

1. Efficient and detailed methods have been worked out for the preparation of synthetic and natural humates containing iron, aluminum, manganese, zinc, nickel, copper, or other similar metals whose inorganic salts are ordinarily highly insoluble over some portion of the physiological range of pH of interest in connection with plant growth.

2. One or two small applications of these humates will ordinarily supply sufficient iron or similar metal to water or sand cultures to last throughout the entire period of growth. The metals in the form of humates are stable in alkaline and neutral as well as moderately acid culture media, and are not precipitated by phosphate.

FERTILIZER INVESTIGATIONS UNIT
BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

LITERATURE CITED

1. BURK, D., LINEWEAVER, H., and HORNER, C. K. Iron in relation to the stimulation of growth by humic acid. Soil Sci. **33**: 413–454. 1932.
2. ———, ———, and ———. The physiological nature of humic acid stimulation of *Azotobacter* growth. Soil Sci. **33**: 455–487. 1932.
3. ———, ———, ———, and ALLISON, F. E. The relation between iron, humic acid and organic matter in the nutrition and stimulation of plant growth. Science n.s. **74**: 522–524. 1931.
4. ERDENBRECHER, A. H. Ueber eigentümliche Zersetzungen von Saccharose und die Verwendung der Zersetzungsprodukte zur Darstellung kolloidal löslicher Metalle. Zeitschr. Ver. deutsch. Zuckerind. **82**: 718–741. 1932.
5. FRAPS, G. S. The effect of additions on the availability of soil potash, and the preparation of sugar humus. Texas Agr. Exp. Sta. Bull. **190**. 1916.
6. JOHNSTON, E. S. The functions of radiation in the physiology of plants. II. Some effects of near infra-red radiation on plants. Smithsonian Misc. Coll. **87**: no. 14. 1932.
7. SPRENGEL, C. Chemie für Landwirthe, Forstmänner, und Cameralisten. Göttingen. 1832.

CHEMICAL CHANGES IN CARROTS DURING GROWTH

HANS PLATENIUS

(WITH FIVE FIGURES)

Introduction

This study was carried out in connection with a general investigation of the factors influencing quality in carrots. It was recognized that age and size of the roots play an important rôle in determining quality. Although it was realized that quality in vegetables, an indefinite term in itself, cannot be measured accurately, it was expected that the analysis for certain chemical constituents would give some indication of changes in eating quality as well as in nutritional value of the carrots.

Since it was necessary to take samples at various stages of the growth period, the plan of the experiment was enlarged so as to gain some information regarding the rate of growth of the plant, the rate at which certain mineral nutrients are absorbed, and various products of photosynthesis are formed.

Materials and methods

The carrots for this study were grown in the gardens of the Department of Vegetable Crops at Cornell University during the summer of 1932. Manure and a 5–10–5 fertilizer were applied to the soil prior to sowing. An overhead irrigation system supplied adequate moisture to the plants during periods of drought. Seed of the Chantenay variety was sown May 1 in rows 18 inches apart. About August 15 the carrots had attained the optimum size for market. Growth of the roots continued until the last day of sampling, October 15, but they were then of such a large size that they would have been considered of inferior quality on the market.

SAMPLING

The first sample was taken June 16, one and a half months after sowing of the seed. Between June 16 and August 15 the carrots were sampled at approximately semi-monthly intervals; after August 15 at monthly intervals. A representative sample of at least twenty individual plants was taken at noon of each sampling day and an aliquot of the composite sample was later used for chemical analysis. The whole plant with the exception of the smaller side roots was pulled and taken at once to the laboratory. Roots and tops were separated and the weight of each was recorded. The material from both lots was then cut into small pieces and mixed thoroughly. An aliquot of 100 gm. was used for the determination of dry weight while another aliquot of 150 gm. was dropped into jars containing sufficient 95

per cent. alcohol to make a final concentration of about 75 per cent. After boiling for 10 minutes the jars were sealed and stored for analysis.

Methods of chemical analysis

(a) Dry weight.—The material was dried in a ventilated oven at 60° C. for 40 hours after the sample had been heated at 100° for one hour to inactivate the enzymes.

(b) Sugars.—A modification of the picric acid reduction method described by Willaman and Davison (8) was used.

(c) Starch.—The extracted material was digested with takadiastase at 38° C. for 20 hours, filtered, and the filtrate clarified with lead acetate, deleaded, and hydrolyzed with dilute hydrochloric acid. Glucose was determined in the hydrolyzed material by the picric reduction method.

(d) Crude fiber.—A slightly modified official procedure was used.

(e) Lignin.—A method which was described in detail by Phillips (6) was used. Lignin was determined gravimetrically after cellulose had been removed by fuming hydrochloric acid. Corrections were made for ash and protein.

(f) Calcium and phosphorus.—These constituents were determined by the official method recommended by the A.O.A.C. (5).

Analytical results

The results of the chemical analyses were expressed as percentages of dry weight, which made it possible to eliminate certain fluctuations in composition caused by variations in the moisture content of the plant. In order to study the relationship between the rate of formation of the various chem-

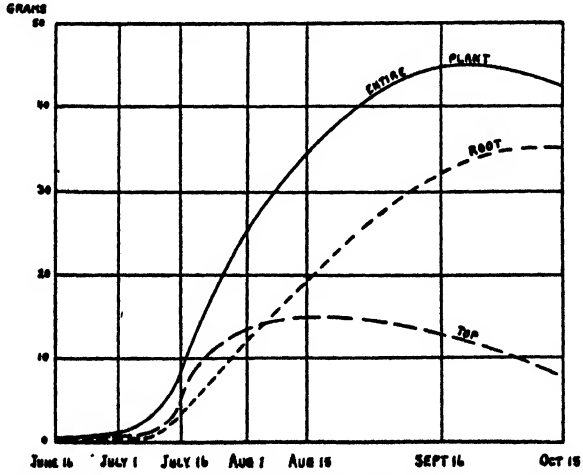


Fig. 1. Rate of growth of entire carrot plant, root, and top in grams of dry weight.

ical constituents analyzed, the data were also calculated as weight per plant or root respectively. A graphic representation of the rate of growth of the root, top, and entire plant is given in figure 1. The various curves do not give a clear conception of the true relative growth rate; a better representation can be obtained when the rate of growth is plotted on a ratio scale (fig. 2), where the paper in the vertical direction is ruled on a logarithmic

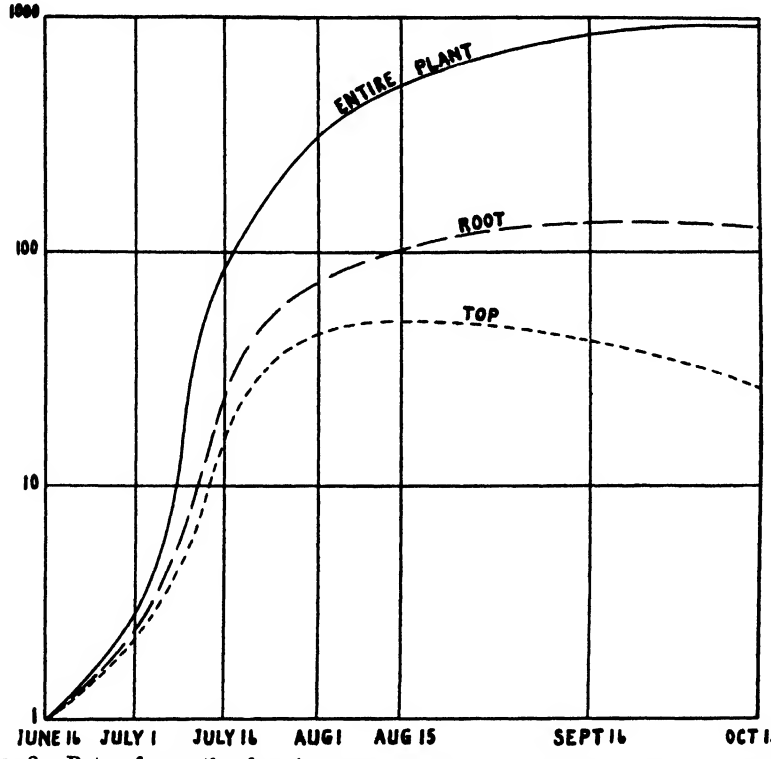


FIG. 2. Rate of growth of entire carrot plant, root, and top. Data (calculated on dry weight basis) are the same as given in fig. 1, but are plotted on the ratio scale.

scale while the horizontal scale gives time in absolute amounts. The nature of the ratio scale is discussed in detail by CHADDOCK (2). The spacings in the vertical direction represent true ratios, the slope of the curve indicates the rate of increase or decrease, and the slopes of different curves are comparable and represent comparative rates of increases or decreases regardless of the absolute values. Only the slopes are comparable, not the levels of the points above the base line. The obvious advantage of using such a scale is to bring several curves close together for comparison and to show the true relationship between relative increases or decreases. The ratio scale has been used by BUCHANAN and FULMER (1) to illustrate the growth rate

of bacteria. Strangely enough, few other workers dealing with growth rates of plants have used this method of presentation.

A few striking facts become apparent when the chemical composition of the root at the various stages of growth is examined with regard to those constituents which ordinarily are considered as factors determining quality.

The moisture content fluctuated somewhat and decreased slightly with age. The decrease in the water content was not more than 2.5 per cent. of the entire fresh weight, however, and with an average moisture content of nearly 90 per cent. it becomes doubtful whether such small fluctuations can materially influence the crispness of the tissue as far as eating quality is concerned.

The total sugar content increased slowly, and on the dry weight basis was about 2.5 per cent. higher in the old carrots than in the young ones (table I and fig. 3). A very definite relation was found between reducing

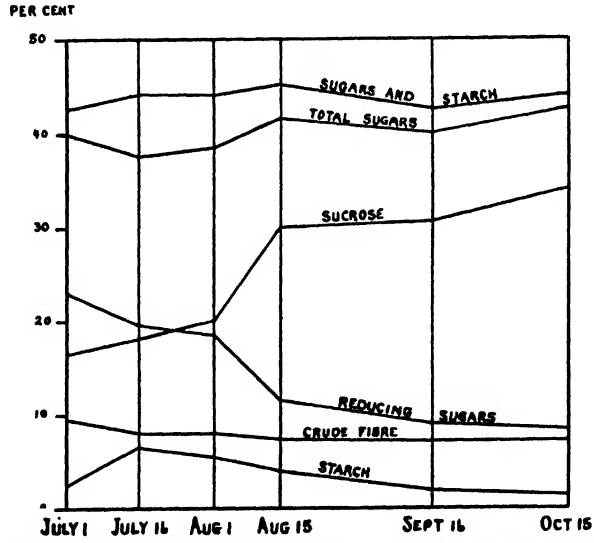


FIG. 3. Chemical composition of carrots at various stages of the growth period. Data expressed as percentages of dry weight.

sugars and sucrose. While the reducing sugars declined steadily, sucrose increased at about the same rate. On a percentage basis reducing sugars dropped to less than one-half of their original value while the percentage of sucrose more than doubled. In order to determine the relative sweetness of carrots at different stages of age it is important to identify the reducing sugars present, since different sugars vary widely in their relative sweetening power. For instance, should the greater part of the reducing sugars be in the form of fructose, young carrots would be sweeter than old ones. On the other hand, if glucose forms most of the reducing sugars the relation

TABLE I

CHEMICAL COMPOSITION OF CARROT ROOTS AND TOPS AT VARIOUS STAGES OF GROWTH. ALL DATA EXPRESSED ON DRY WEIGHT BASIS

DATE	DRY WEIGHT	AVERAGE WEIGHT OF PLANT	SOLUBLE NITROGEN	PROTEIN NITROGEN	TOTAL NITROGEN	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	CRUDE FIBER	LIGNIN	CaO	P_2O_5
ROOTS	%	*gm.*	%	%	%	%	%	%	%	%	%	%	%
June 16	10.04	0.037											
July 1	11.34	0.1066	0.580	1.234	1.814	23.24	16.53	39.77	2.52	9.50	2.98	0.066	1.356
July 16	12.42	3.116	0.436	0.914	1.350	19.32	18.20	37.52	6.62	7.94	2.81	0.094	0.919
Aug. 1	11.12	11.70	0.612	0.803	1.415	18.52	20.05	38.57	5.47	7.83	2.95	0.078	0.933
Aug. 15	11.38	19.17	0.481	0.861	1.342	11.53	29.82	41.35	3.89	7.63	2.49	0.080	1.004
Sept. 16	12.50	32.11	0.674	0.786	1.460	9.86	30.36	40.22	2.05	7.24	1.83	0.069	1.121
Oct. 15	12.44	34.82	0.621	0.766	1.387	8.46	33.91	42.37	1.48	7.30	1.95	0.061	1.031
TOPS													
June 16	14.17	0.2996											
July 1	16.53	0.6771	0.471	0.309	0.780	2.81	5.34	8.15	0	9.04	5.18	3.37	1.158
July 16	16.13	4.655	0.286	0.261	0.547	6.57	1.48	8.05	0	10.60	6.82	3.36	0.664
Aug. 1	16.95	13.57	0.233	0.189	0.412	7.91	1.99	9.90	0	11.03	6.50	3.23	0.778
Aug. 15	17.62	15.14	0.174	0.198	0.372	6.87	1.52	8.39	0	10.47	8.13	3.59	0.724
Sept. 16	19.39	13.04	0.158	0.224	0.382	3.12	1.70	4.82	0	12.24	11.21	4.93	0.683
Oct. 15	21.96	7.879	0.170	0.208	0.378	2.70	1.53	4.23	0	13.44	10.73	4.57	0.673

would be reversed. The isolation and identification of certain sugars in a mixture is a difficult procedure. PINOFF and GUDE (7) describe a color test using ammonium molybdate, which is fairly specific for fructose. Using this test, only traces of fructose could be found in the mixture of sugars extracted from young carrot roots. Assuming that all reducing sugar is present as glucose, older carrots should be 19 per cent. sweeter than young ones when the relative amounts of these sugars present is considered. This agrees very well with the fact that old carrots were found to be noticeably sweeter to taste than young roots. This fact conforms with the statement of HASSELBRING (3) who considers the natural content of sucrose as determining largely the flavor in carrots.

The percentage of starch increased very rapidly at first to 6.6 per cent., then declined slowly to 1.5 per cent. of the dry weight.

Very surprising is the fact that the amount of crude fiber, which was expected to increase with age, actually declined. The same holds true for lignin, which occurs in small amounts particularly around the tracheae.

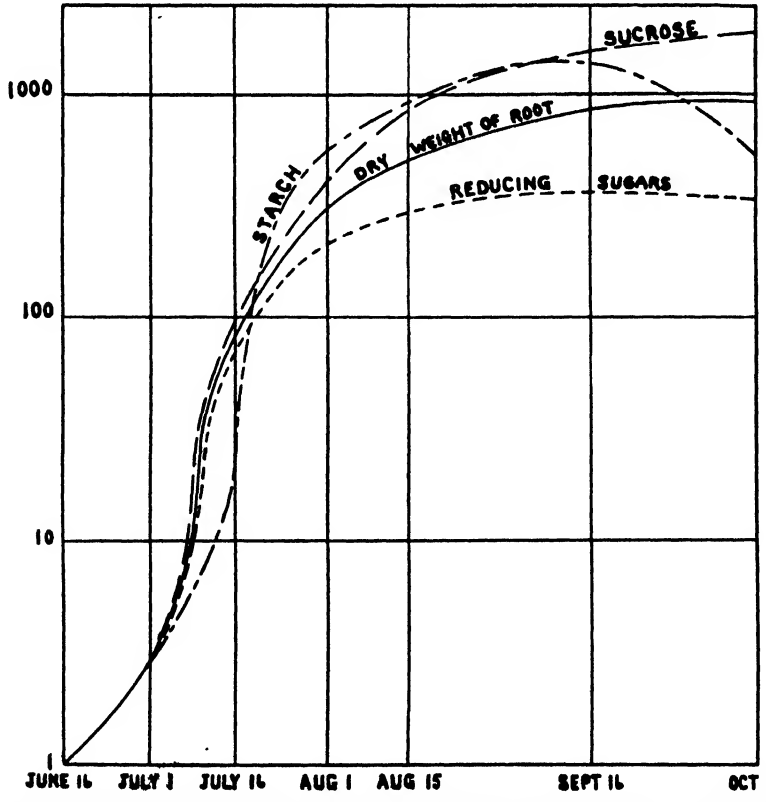


FIG. 4. Rate of accumulation of carbohydrates in carrot roots. Data plotted on ratio scale.

When the growth curves for the roots and tops are considered separately it is found that their rates of growth differ widely (fig. 2). While the tops had ceased growing entirely August 15, the roots were still gaining in weight two months later. The tops actually lost some of their dry matter after August 15. This loss was partly due to respiration and the slow dying off of the outer leaves. It can safely be assumed, however, that the principal loss was due to translocation of sugars from the leaves to the roots. In this connection it is interesting to notice that no trace of starch was found in the leaves at any time.

Figure 4 shows that reducing sugars accumulated in the roots at a much slower rate than sucrose, which necessarily resulted in a much lower percentage of glucose than sucrose at the end of the storage period. The formation of starch in the roots was most rapid during July, the relative rate of increase being greater than that of the total dry weight or of the sucrose content in the roots in this period. During the last month, from September 15 to October 15, there was a sharp drop in the starch content. Apparently, the starch was reconverted into sucrose, which continued to accumulate until the last day of sampling.

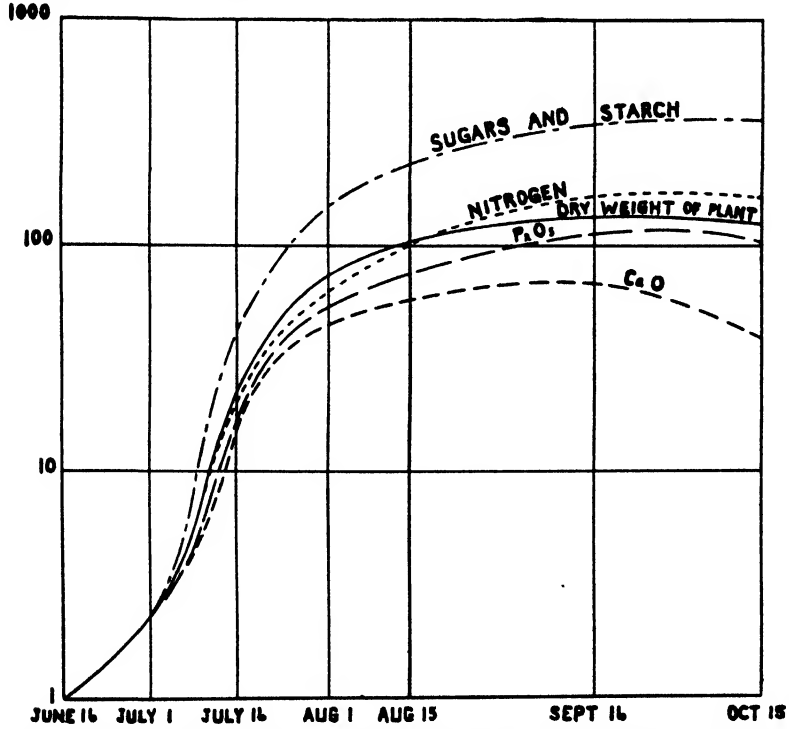


FIG. 5. Rate of absorption of calcium, phosphorus, and nitrogen and rate of accumulation of starch and sugars in the entire plant. Data plotted on ratio scale.

In figure 5 is shown the relative rate of the absorption of some mineral constituents, calcium, phosphorus, and nitrogen, by the entire plant. It is obvious that the relative rate of absorption of minerals is much greater at the beginning of the growth period than at the end when compared with the accumulation of photosynthetic products. During the last stage some of the mineral and nitrogen constituents actually disappeared from the plant, probably through the dying off of the outer leaves. It is doubtful whether any minerals actually migrated out of the plant through the roots. The more rapid loss in calcium as compared with the loss in phosphorus or nitrogen is explained by the fact that the tops contained four to seven times as much calcium as the roots, while phosphorus and nitrogen were distributed more evenly through the entire plant.

Discussion

There is a general assumption among consumers that small young carrots are far superior to large old ones, which is reflected by the fact that young carrots bring a much higher price on the market. The present study makes it extremely doubtful whether this assumption is justified. The analytical data show clearly that sweetness in carrots, which probably is controlled primarily by their sucrose content, increases markedly with age.

In the present study no data were obtained which support the general idea that young carrots are more tender than old ones. On the contrary, the amount of crude fiber was found to decrease slightly with age. Several persons in the Department of Vegetable Crops, Cornell University, who compared young and old carrots with respect to tenderness, agreed that no significant difference could be observed between the two groups. If a slight difference was noticed it was always in favor of the old ones. Chemically and microscopically no change in the amount of lignified matter could be found. In older carrots, however, the cells of the outermost cortex are very much smaller, which brings the cell walls closer together and consequently the skin is slightly harder than in young carrots. Under certain conditions it may be found that young carrots are more crisp than old ones, especially if the latter group has been kept in storage for some time. Crispness is controlled by the water content of the roots and the turgidity of the cells.

The color of the carrots as determined by the amount of carotene present improved decidedly with age. Although no measurements of the relative amounts of carotene present were made, the difference in color was striking and consistent. Since it is a generally accepted theory that carotene and vitamin A content are closely connected, this would suggest the possibility that the vitamin A content in old carrots is higher than in young ones. The percentage of dry matter and combined sugars and starch was shown to

increase slightly with age while the amount of crude fiber decreases. This indicates that the food value of carrots tends to increase rather than decrease with age.

HASSELBRING (3) concluded from his data that starch is absent in carrots, although he cited the work of other investigators who reported its presence. The writer was able to verify the occurrence of starch by identification of starch grains in the dried root tissue.

The curves obtained for the rate of growth and the rate of accumulation of photosynthetic products are extremely smooth, especially if it is realized that this experiment was carried out in the field under variable weather conditions.

The rôle which the root plays as a storage organ is well illustrated. For two months after the tops had ceased growing, translocation of carbohydrates from the tops to the roots continued.

Little work has been done on the rate at which mineral constituents are absorbed by vegetables. Such studies may be of importance in determining the proper time at which fertilizer can be applied to the plants to best advantage. KOTOWSKI (4) made such a study for several vegetables. However, he took only three samples during the entire growing period, and this can hardly be considered sufficient to give accurate data on the rate at which nutrients are absorbed. The present results show that all minerals had been absorbed by the plant at least a month before the roots obtained their maximum size.

Summary

1. Data are given which indicate that carrots lose neither in eating quality nor in food value as they grow older. The sucrose content, which is considered to determine flavor in carrots, rises decidedly while the percentage of crude fiber becomes slightly lower.

2. Older carrots are better colored owing to their higher carotene content.

3. The occurrence of small amounts of starch in carrot roots was verified. The tops were found to be free of starch throughout the growth period. Curves are presented of the growth rate of roots and tops and the rate of accumulation of various chemical constituents in the plant.

CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. BUCHANAN, R. E., and FULMER, E. I. Physiology and biochemistry of bacteria. 3 Vol. Baltimore. 1928–30.
2. CHADDOCK, R. E. Principles and methods of statistics. Cambridge, Mass. 1925.

3. HASSELBRING, HEINRICH. Carbohydrate transformations in carrots during storage. Plant Physiol. **2**: 225–243. 1927.
4. KOTOWSKI, FELIX. Untersuchungen über Verlauf der Nahrungsaufnahme bei Kohlkopf, roter Rübe, Mohrrübe und Zwiebel. Gartenbauwiss. **4**: 484–496. 1931.
5. METHODS OF ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 2d ed. Washington, D. C. 1925.
6. PHILLIPS, MAX. The quantitative determination of methoxyl, lignin, and cellulose in plant materials. Jour. Amer. Off. Agr. Chem. **15**: 118–131. 1932.
7. PINOFF, E., and GUDE, K. Nachweis der Fruktose mit Ammonium Molybdätlösung. Abderhalden: Handbuch der biologischen Arbeitsmethoden, Abt. 1, Teil **5**: 87–88.
8. WILLAMAN, J. J., and DAVISON, F. R. Some modifications of the picric acid method for sugars. Jour. Agr. Res. **28**: 479–488. 1924.

APPLICATION OF QUANTITATIVE SPECTRAL ANALYSES TO BINARY MIXTURES OF THE COMMON CAROTENOIDS

ELMER S. MILLER[1]

The application of quantitative spectral analyses to biological compounds was first reported by KUHN and SMAKULA (1) in 1931. By trial and error, they varied the amounts of each component in solution until the resultant absorption curve was identical with that obtained for the unknown mixture. At best, this method is limited in its application. The accurate spectro-photoelectric method described by ZSCHEILE, HOGNESS, and YOUNG (2) has been used to obtain the quantitative data presented in this paper.

From Lambert's law, we obtain this relationship[2] (ZSCHEILE, 3):

$$\log \frac{I_o}{I_x} = \alpha c\, x \tag{1}$$

When two components are present, the *effective* absorption coefficient β_T (for the total number of components) is defined by the following consideration:

Let:

$$\beta_1 = \alpha_1 c_1 \text{ and } \beta_2 = \alpha_2 c_2$$

$$c_T = c_1 + c_2 \text{ (the total concentration)} \tag{2}$$

and

$$\beta_T = \alpha_T\ c_T \tag{3}$$

then

$$\beta_T = \beta_1 + \beta_2 = \alpha_1\, c_1 + \alpha_2\, c_2 = \alpha_T\ (c_1 + c_2) \tag{4}$$

From ZSCHEILE (3)

$$\log \frac{I_o}{I_x} = \beta_T x = \alpha_T c_T x \tag{5}$$

If for some wave length, λ', $\alpha_1' = \alpha_2'$

[1] National Research Fellow in the Biological Sciences.

[2] In the equations superscripts refer to wave lengths and subscripts to the different components, and

I_o = intensity of light transmitted by solvent-filled cell.
I_x = intensity of light transmitted by solution-filled cell.
x = thickness of absorption cell (4.25 cm.).
β = absorption coefficient.
α = specific absorption coefficient.
c = concentration in gm. per liter.

then

$$\beta_T' = \alpha_1' (c_1 + c_2) \qquad (6)$$

Thus when β_T is measured at λ', and α_1 and α_2 are known, c_T may be calculated.

The relative and absolute amounts of components 1 and 2 can be determined by measurement of β_T'' for some other suitable wave length λ'' for which there is an optimum difference between α_1 and α_2; thus

$$\beta_T'' = \alpha_1'' c_1 + \alpha_2'' c_2 \qquad (7)$$

Hence, c_1 and c_2 can be determined by solving equation (2) and equation (7).

It is not necessary to use a wave length for which $\alpha_1 = \alpha_2$. In general, c_1 and c_2 can be determined by solving simultaneously two equations like (7) obtained for two suitable wave lengths.

Accurate analyses of binary mixtures of the carotenoids

Before the analyses of binary mixtures can be undertaken, it is necessary to determine the absorption coefficients of the pure components (fig. 1, Plant Physiol. 9: 693. 1934). These data permit the selection of the suitable wave lengths of light to be employed in the analyses.

Since the total concentration of the mixtures was known (1.2 mg./L.), β_T was not measured at a wave length at which components 1 and 2 absorb equally. In figure 1,[3] it is seen that both the carotene isomers absorb equally at 4450 Å. U. Hence, from experimentally determined values of β_T at this wave length, it is possible to calculate the total concentration $(c_1 + c_2)$ by equation (6). The percentage composition of each component present can then be determined by the measurement of β_T'' (equation 7). For the purpose of checking upon each other, two or more wave lengths were employed. The standard solutions were made up with an accuracy of less than 1 per cent. error.

The effective absorption coefficient, α_T, for known mixtures was plotted against compositions. This gives a straight line relationship which may be used in the analyses of unknowns (ZSCHEILE, 3). This straight line relationship also indicates that Beer's law holds for the concentration employed.

α_T is obtained from measurements for which equation (6) is employed. The following equation (8) shows analytically that such a relationship $\left(\frac{c_1}{c_1 + c_2}\right)$ should exist. From equation (4) we obtain,

$$\alpha_T = \alpha_1 \frac{c_1}{c_1 + c_2} + \alpha_2 \frac{c_2}{c_1 + c_2} = \alpha_1 \frac{c_1}{c_1 + c_2} + \alpha_2 \left(1 - \frac{c_1}{c_1 + c_2}\right)$$

[3] In this study, the same solvent was employed as in Plant Physiol. 9: 693–694. 1934, and reference 4.

or

$$a_T = (a_1 - a_2)\frac{c_1}{c_1 + c_2} + a_2 \qquad (8)$$

The percentage composition of mixtures of alpha and beta carotene, beta carotene and lycopene, and lycopene and leaf xanthophyll were calculated from the experimental data at the various wave lengths by employing equation (8) and the final values of a_1, and a_2 (of the pure components), as presented in fig. 1. The percentage composition for the binary mixtures of the carotenes are summarized in Table 1. The greatest deviation from the true composition in any mentioned binary mixture is 0.8 per cent. and the accuracy of this method may be seen by a comparison of the first and last rows of figures.

TABLE I

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF ALPHA AND BETA CAROTENE IN 20.0 PER CENT. ETHER AND 80.0 PER CENT. ETHANOL

		Composition in terms of beta carotene								
Known composition (per cent.)		5.0	10.0	25.0	50.0	75.0	90.0	95.0	Extract of analyses in samples	
	Wave length	%	%	%	%	%	%	%	*I*	*II*
Quantitative	4636 A. U.	5.3	9.8	25.2	50.2	75.2	89.9	94.5	69.2	69.3
spectral	4862 A. U.	4.8	9.9	24.9	49.8	74.8	90.2	95.2	69.7	69.1
analyses	4956 A. U.	5.4	10.1	25.1	50.0	74.8	90.3	94.8	67.5	68.2
Av. per cent. composition		5.2	9.9	25.1	50.0	74.9	90.1	94.8	68.8	68.9

Analyses of unknowns

Columns I and II in table I contain the analyses of solutions of unknown composition. Determinations for the three wave lengths agree within 1 per cent. error. This method is now employed for the quantitative analyses of the carotenoids present in the grasses with about the same degree of accuracy. Thus, by this spectro-photoelectric method, it is possible to analyze unknown binary mixtures of the carotenoids and to determine the total concentration and the percentage composition with an error of about 1.0 per cent.

Summary

1. Quantitative spectral analyses have been applied to binary mixtures of the common carotenoids. The percentage composition of binary mixtures of the carotenoids were determined with an error of about 1 per cent.

2. Over a limited range of concentration, it has been shown that Beer's law is obeyed by binary mixtures of carotenoids.

The writer wishes to express his appreciation to Prof. T. R. HOGNESS for suggestions and invaluable criticism during the progress of this investigation, and to Dr. F. P. ZSCHEILE for his valuable criticism of the paper.

GEORGE HERBERT JONES CHEMICAL LABORATORY
THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. KUHN, RICHARD, and SMAKULA, ALEXANDER. Spektrophotometrische Analyse des Eidotterfarbstoffes. Zeitschr. physiol. Chem. **197**: 161–166. 1931.
2. ZSCHEILE, F. P., JR., HOGNESS, T. R., and YOUNG, T. F. The precision and accuracy of a photoelectric method for comparison of the low light intensities involved in measurement of absorption and fluorescence spectra. Jour. Phys. Chem. **38**: 1–11. 1934.
3. ZSCHEILE, F. P., JR. A quantitative spectro-photoelectric analytical method applied to solutions of chlorophylls a and b. Jour. Phys. Chem. **38**: 95–102. 1934.
4. MILLER, ELMER S. Improved methods for the purification of the common carotenoids, and the quantitative measurement of their absorption spectra. (To appear in an early issue of the Bot. Gaz.)

WHAT THE ORGANIC CHEMIST CAN DO FOR PLANT PHYSIOLOGY[1]

HUBERT BRADFORD VICKERY

It is not customary for addresses made to scientific gatherings to be founded upon a text after the manner of a sermon. Notwithstanding this, I am going to use a text on the present occasion. A few years ago a valuable little book appeared entitled "Recent Advances in Plant Physiology," written by E. C. BARTON-WRIGHT, of King's College, London.[2] In the preface to the book the author makes the following statement:

"Our present knowledge of plant metabolism is very seriously deficient in several directions. This unfortunate state of affairs is due to several causes, among the principal of which is the fact that in plants several complex chemical reactions take place within the compass of a single cell, which makes the matter difficult of investigation. Nevertheless, the difficulty is intensified by the sporadic invasions of organic chemists into a domain of which they have little or no knowledge, with ready-made explanations based on *in vitro* experiments which are probably remote from the chemical reactions of the living plant. It is difficult to know why botanical physiology should be made the general playground for the imaginative theorising of persons who have no very reliable knowledge of the living plant."

It seems to me that the occasion of a STEPHEN HALES address by one who is frankly a chemist, and who certainly lays no claim to a "reliable knowledge of the living plant" presents an opportunity for a defense of the custom imputed to us chemists of straying into places where we do not belong, and where apparently we are not universally welcomed.

Has the organic chemist anything to offer to plant physiology that entitles him to expect a welcome? One of the greatest of recent plant physiologists, evidently believes that he has. The late KOSTYCHEV writes,[3] "Physiology endeavors to trace the phenomena of life to the general laws of chemistry and physics, *i.e.*, in the final analysis, to state all the chemical processes in organisms by chemical equations, and to interpret the energy transformations and the entire structural features on the basis of physical laws."

Let us consider this view of the aims of physiology in a little detail. The plant absorbs its nutriment from the soil in the form of an aqueous solution of inorganic ions, the most important being nitrate, ammonium, phosphate,

[1] Third STEPHEN HALES address, read at the Boston meeting, December 29, 1933

[2] BARTON-WRIGHT, E. C. Recent advances in plant physiology. Blakiston. Philadelphia. 1930. (p. vi.)

[3] KOSTYCHEV, S. Chemical plant physiology, translated by C. J. LYON. Blakiston. Philadelphia. 1931. (p. xiii.)

and potassium, with magnesium, calcium and a number of others of less significance only inasmuch as they are needed in smaller amount. Its carbon it takes directly from the air as carbon dioxide. From these extremely simple components the cells manufacture all that is required for the growth and reproduction of a highly complex organism. No one can state the number or the identity of the organic substances in a plant cell. We know in a general sort of way that the cell contains proteins, carbohydrates, fats, lecithins, sterols, hydrocarbons, hydroxy-acids, amino-acids, peptides, amides, purines, betaines, quaternary bases such as choline, to mention some of the better recognized types of substances; in addition there are enzymes —substances probably of protein nature which act as catalysts of the myriads of reactions that occur—and inorganic salts.

As I see it there are two main groups of plant physiologists—those who are chiefly interested in such topics as plant movements in response to various stimuli, in the response of the plant to changes in illumination, or of temperature, or of the composition of the air surrounding it, or of the soil solution that bathes its root; in short those who are concerned with the behavior of the plant unit as a living organism. But there are also many who are interested in the detailed problems of plant function, in the means by which it absorbs its food, and what it does with it, in the mechanism whereby it transports the elaborated products derived from the food substances, in its capacity for food storage and subsequent utilization, in its response to adversity, or injury. Such people have an insatiable curiosity to discover "how it works," and it is to these that the chemist can render his chief service.

I wish to discuss two of the functions that the chemist fulfils for these persons. He can supply them with methods, and he can supply them with critique—both of these functions are of far-reaching importance.

Measurement is a primary requisite to real advance in knowledge. The human mind responds most eagerly when a statement is couched in quantitative terms. We may be interested to learn that business is better, but the information that the bank clearings have increased by such and such a sum during the past week is far more meaningful. It is of more significance to state that a leaf, when placed in water, has increased in weight by such and such a per cent., than to state that it has become more turgid.

The function of the chemist is to supply methods whereby accurate measurements of the composition of tissues can be made. It is his responsibility to see to it that his method shall be specific and, where this is difficult or impossible, to provide for controls and corrections whereby the effect of interfering substances is minimized. It is his further responsibility to see to it that his method is accurate and reliable. In addition it is thoroughly desirable that his method shall be rapid and convenient. Methods that combine

all three features of specificity, reliability and convenience will occur to all of you. Some of these methods, such as the Kjeldahl method, have become integral parts of the equipment of every laboratory; others, such as the quinhydrone method for hydrogen-ion activity measurements, are now very commonly employed, while still others, such as an extensive group of colorimetric methods which require the use of a spectrophotometer, are only beginning to make their way into physiological laboratories. Many methods require the development of special equipment for their convenient application. This has certain disadvantages, especially when funds are limited, but the psychological effect is usually good. One approaches a piece of new apparatus with a species of awe and, after he has turned a stop-cock at the wrong moment a few times, this may change to annoyance; but finally, when he has compelled that inanimate object to tell him the truth again and again, his feeling becomes tinged with respect, and he acquires a sense of mastery over nature that is not without its own peculiar reward.

This brings me to another point. One may develop a method that has all desirable features, and describe it in full detail, but no control can be exercised over what the users of the method may do with it. There is probably no method so simple that it does not have to be learned by repeated trial. Even a chemist gets out of practice in weighing at the ordinary analytical balance; a modern microbalance requires a ritual that has to be painfully acquired. No one can expect to take a new method into the laboratory and apply it without going through a long series of control tests on known amounts of the material under study, together with blank tests to discover if his particular set of reagents is above reproach. Furthermore there is no justification for dismissing a new method that has failed to work until one is certain that he has followed directions implicity and exactly. The converse of these propositions is equally important. No chemist should publish a method until he has made certain, by many tests, that it will do what he claims for it, and his obligation for concise and accurate description is, of course, supreme.

I wish to turn now for a moment to the other important function that I think the chemist can fulfil in his connection with plant physiology, namely, that of criticism. It is always interesting to read of the advance that someone has made in unraveling the chemical reactions that take place in the living cell. But there is also the obligation to examine the method by which the results were obtained to see if it really is capable of yielding an unequivocal answer. May I cite an example? A few years ago it was noted in our laboratory,[4] that the quantity of ammonia yielded by mild acid hydrolysis of extracts of tobacco leaf varied widely with the concentration of the acid, if hydrochloric acid were employed. The procedure was designed to determine the quantity of amide nitrogen in the extracts; the answer pro-

[4] VICKERY, H. B., and PUCHER, G. W. Jour. Biol. Chem. 90: 179–188. 1931.

vided by the experiment was that the quantity varied according to the strength of the reagent—a manifestly absurd result. The difficulty was soon traced to the nitrate which is present in unusually high concentration in tobacco leaf extracts. This reacted with the hydrochloric acid and some easily oxidized constituent of the extract to yield ammonia. The difficulty disappeared when we employed sulphuric acid. As a result of this experience I always note carefully the reagent that is employed for the hydrolysis of amides when I see a paper on this subject. If the author used hydrochloric acid, and failed to demonstrate the absence of nitrate, I am compelled to discount the accuracy of his determinations and consequently of his conclusions as well.

A rather striking example of the necessity for critical examination of published results occurs in the splendid review of organic acid metabolism recently published by BENNET-CLARK.[5] The author quotes a great deal of experimental work on the change in the acid content of leaf tissues when these are placed under various conditions. He discusses the changes as if they were due to variations in the proportion of malic acid, although what was measured was the titratable acidity. He may be correct in his assumption, but there is no demonstration of this. It is certain that the titratable acidity changed, but there is no certainty that the malic acid was responsible—this is pure assumption, consequently the theoretical interpretation loses much of its force. BENNET-CLARK himself draws attention to the necessity for critical examination of experimental conditions. He points out that many of RAISTRICK'S supposedly aerobic mold cultures were really grown for part of the time under anaerobic conditions inasmuch as air was circulated over the solution in the plugged flasks only once a day.

Considerations such as these impel me to believe that the plant physiologist can ill afford to dispense with the coöperation of the chemist. In fact I am prepared to go further. If plant physiology proposes to advance beyond the stage of a purely descriptive science, if it is really the aim of this science to account as far as possible for the phenomena of life, and growth, and reproduction in the plant world without resort to vitalistic hypotheses, there is no question in my mind but that the attack on the problem must be made by chemical methods.

The major obligation in life of many, perhaps most, of my audience is that of teaching. You have in your hands the preparation of the younger workers for the investigations that lie ahead. Allow me to urge you to place the greatest emphasis of which you are capable upon the necessity for thorough preparation in chemistry. I should subordinate this only to the training in that most difficult of all human activities, clear and logical thinking.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

[5] BENNET-CLARK, T. A. New Phytol. 32: 32–71, 128–161, 197–230. 1933.

A LARGE-CAPACITY DRYING OVEN WITH CONSTANT UNIFORM TEMPERATURE AND FORCED VENTILATION

EMMETT V. MARTIN

(WITH ONE FIGURE)

Temperature and rate of drying are important factors in desiccation of plant material for analysis. Various temperatures, ranging from 30° to 105° C., have been used for this purpose, although there is no particular one which is entirely satisfactory in all respects. In the higher range there may be a decrease of soluble nitrogen by coagulation, and caramelization of the sugars may occur, while at the lower values there will be enzyme action and respiration. TOTTINGHAM and LINK[1] have made a special study of methods of desiccation of plant tissue, and have given data showing that preparation under widely different conditions yields results that are not comparable. They conclude that material which dries readily undergoes least destructive change with a temperature of about 65° C. When the material is dried in an oven with very little ventilation, the humidity soon becomes so high that drying proceeds very slowly, thus allowing destructive action to continue for a longer period of time. If one wishes to compare analyses of stems and leaves, the former should be sliced longitudinally into slender strips so that they will dry more quickly. Leaves also can be cut into a number of pieces to similar advantage. When the material is placed in the oven, it should be well spread out to allow good circulation of air around all parts.

In the preparation of plant tissue for analysis, therefore, it is desirable to use an oven that has a fairly constant uniform temperature and good ventilation. Furthermore, if considerable quantities are to be dried, a rather large capacity is needed. Such ovens are obtainable, but the expense may in some cases be prohibitive. It is the purpose of this paper to describe an oven that can be constructed by local sheet-metal workmen at much lower cost than its equivalent can be purchased from manufacturing companies.

In figure 1 is shown a diagram of a cross-section through the center of the oven, drawn to scale. It is made up of three separate sections, *A*, *B*, and *C*. Section *A* comprises the oven proper, *C* contains the heaters, and *B* provides extra insulation between *A* and *C*. The outside dimensions of

[1] LINK, K. P., and TOTTINGHAM, W. E. Effects of the method of desiccation on the carbohydrates of plant tissue. Jour. Amer. Chem. Soc. 45: 439–447. 1923 (also 47: 470–476. 1925).

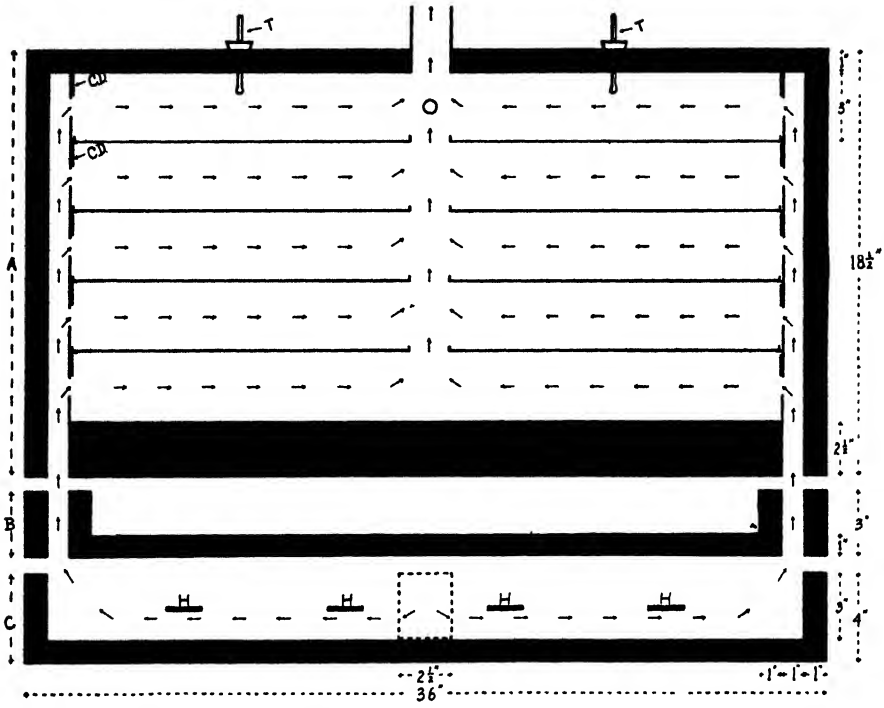


FIG. 1. Diagram of cross-section through center of oven, drawn to scale.

section A are $36'' \times 20'' \times 18\frac{1}{2}''$, the inside are $32'' \times 18'' \times 15''$, while others are shown in the illustration.

The walls are constructed of no. 26 galvanized sheet-iron of two layers 1" apart, except on the floor of section A where the spacing is $2\frac{1}{2}''$. The spaces between layers are filled at the rate of 12 pounds per cubic foot with loose rock wool, manufactured by the Gimco Rock Wool Products Co. The four shelves in section A are made of no. 24 galvanized sheet-iron, with the edges bent over $\frac{1}{4}''$ for added strength. In the center of each shelf and in the top of the oven there is a slot $1\frac{1}{2}'' \times 16''$ to allow for the passage of air. The shelves rest on slides at each end of the oven and are removable.

The amount of air that passes over each shelf is controlled by a diaphragm opening at each end (fig. 1). These openings are $\frac{3}{4}'' \times 16''$ and can be partially or completely closed by means of covers (C, D). These covers are adjustable and are held in place by means of a thumbscrew in a slot at each end.

Heat is furnished by four coils (H) of no. 26 B. & S. gauge chromel A wire wound on strips of transite $\frac{1}{4}'' \times 1\frac{1}{2}'' \times 18''$, supported in place by angle irons. The two outer coils are connected in series and draw 405 watts from a 110-volt A.C. source, while the two inner ones, also in series, take 235

watts. When the oven is in operation the 405-watt coils are heating continuously, but the 235-watt coils are controlled by a DeKhotinsky bimetallic thermoregulator which projects through the rear wall into the center of the space above the top shelf (shown as small circle in figure 1). Electrical connections to the heaters are made through binding posts insulated from the walls by fiber tubing and washers.

Ventilation is produced by a no. 1 baby conoidal blower manufactured by the Buffalo Forge Co. This blower is mounted on a small platform attached to the rear of section *C*. Air is passed from the blower to this section through a short metal tube, which is round at the blower end and square at the oven end. In this tube is mounted an adjustable damper for the control of air flow. The blower has a capacity of 78 cubic feet of air per minute, but its delivery is limited in this case to approximately 12 cubic feet per minute. With this rate of flow the air in the oven is replaced more than twice per minute.

The course of air through the oven is indicated in the figure by small arrows. Leakage between the sections is prevented by padding the points of contact with asbestos, and the sections are prevented from slipping on each other by flanges. The diaphragm covers (*C, D*) are adjusted so that approximately the same amount of air passes over each shelf. To accomplish this, the top covers are nearly closed and the bottom ones wide open; the openings of the others are graduated accordingly.

Between sections *A* and *B* is an air space open at the front and back to the outside. This space and the thick floor of *A* effectively prevent any transfer of heat from the heaters through the floors to the lower shelf. This is necessary in order to obtain uniform temperature in the oven.

Measurements of the temperature distribution within the oven were made by means of six thermocouples operating simultaneously at different points. Readings were taken every few minutes over a complete cycle of opening and closing of the thermostat contacts. The couples were then moved to other points and further readings taken. This process was repeated until all regions of the oven had been covered. The maximum difference in temperature found was 7° C., and its variation at any particular point was not more than 2° C. from the mean at that point. During these tests the thermostat was set at approximately 65° C., registered by thermometers marked *T* in figure 1.

Summary

1. For preparation of plant tissues for analysis, it is desirable to have a drying oven of fairly constant uniform temperature, good ventilation and large capacity.

2. The oven described has the following advantages and characteristics:

a) It can be made by local sheet-metal workmen at much lower cost than its equivalent can be purchased from manufacturing companies.
b) It has a volume of 5 cubic feet and a shelf space of 20 square feet.
c) No two points differ in temperature by more than 7° C.
d) The temperature at any particular point does not vary more than 2° C. from the mean at that point.
e) The air is replaced more than twice per minute.
f) Its maximum power requirement is 640 watts.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

BRIEF PAPERS

QUANTITATIVE ABSORPTION SPECTRA OF THE COMMON CAROTENOIDS[1]

(WITH ONE FIGURE)

The specific absorption coefficients in the visible spectrum have been determined for alpha carotene, beta carotene, leaf xanthophyll, and lycopene[2] in solution. These carotenoids were prepared by the best available

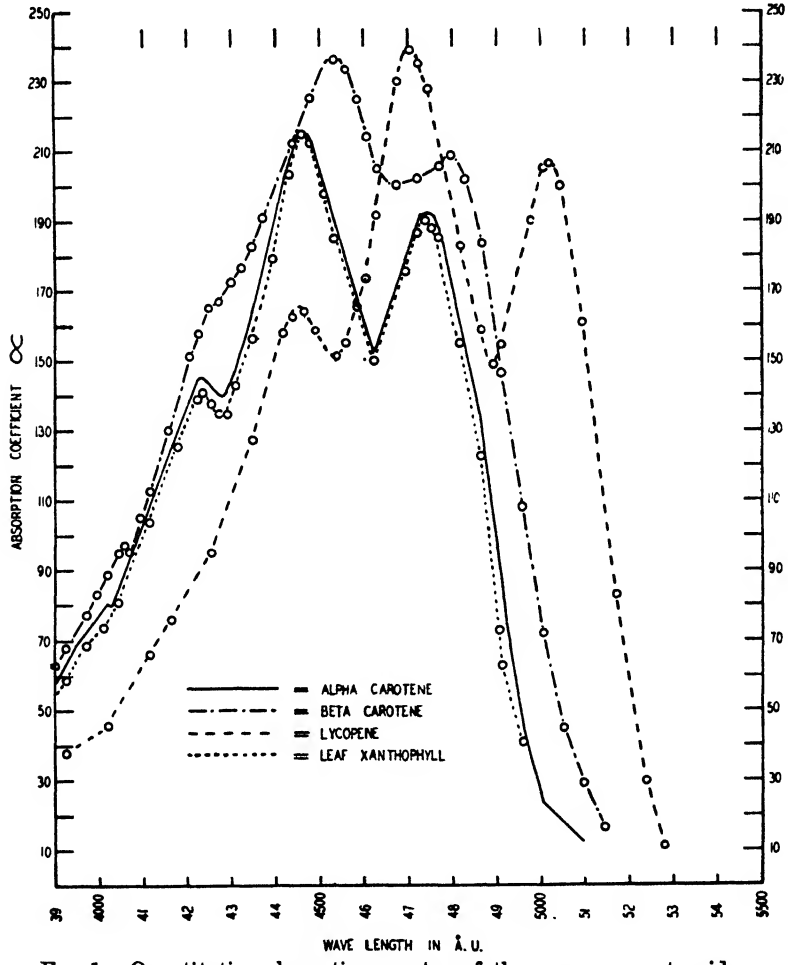


FIG. 1. Quantitative absorption spectra of the common carotenoids.

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] Lycopene sample was purified and furnished by M. B. MATLACK, Bur. of Chem. and Soils. United States Department of Agriculture.

chromatographic methods, care having been taken to exclude oxygen in the important purification processes. The specific absorption coefficients (fig. 1)[3] were later used as the standards of purity of the various samples employed. This method being more sensitive and therefore more reliable than either of the methods employing the melting-point or optical rotation—although the latter two were used in the earlier stages of purification.

The solvent employed was 80 per cent. absolute ethanol and 20 per cent. anhydrous diethyl ether. All of these carotenoids were sufficiently soluble in this solvent. The specific absorption coefficients were determined with an accuracy of less than 1.3 per cent. error, by a method (Jour. Phys. Chem. **38**: 1–11. 1934.) which employs the photoelectric cell to determine the light intensities.

By selecting suitable wave-lengths (fig. 1), accurate quantitative spectral analyses of ternary mixtures of alpha and beta carotene or leaf xanthophyll, beta carotene, and lycopene have been made with an error of less than 2.5 per cent. It is expected that ultimately a procedure will be developed whereby all the photosynthetic pigments may be analyzed in the presence of each other by this optical method.—ELMER S. MILLER,[4] *University of Chicago.*

[3] In Plant Physiol. 9: 179. 1934: It is obvious that the carotene with the maxima farthest toward the infra-red should be beta carotene instead of alpha carotene.

[4] National Research Fellow in the Biological Sciences.

NOTES

Annual Election.—The secretary-treasurer, Dr. A. E. MURNEEK, has announced that the Society has chosen Dr. BURTON E. LIVINGSTON, Johns Hopkins University, as its president for 1934–1935. The vice-president elect is Dr. JAMES P. BENNETT, University of California. This happy outcome of the election insures an auspicious inauguration of the Society's second decade of service to botanical science. All members will wish to enter into active cooperation with the officers to plan and to achieve ever more important objectives.

Pittsburgh Meeting.—Plans for the Pittsburgh meeting are being formulated by the program committee. Every member who can do so should make arrangements to attend this meeting. It is the eleventh annual meeting of the Society, and is centrally located for the eastern and middle western members. The meetings have become more and more valuable each year, so that one can hardly afford to miss them. When the officers call upon members for service in connection with the meetings, it is hoped that no one will refuse to share in the responsibilities, privileges, and duties of membership. As full information as possible will be carried in the October number of PLANT PHYSIOLOGY.

Berkeley Meeting.—The summer meeting held in connection with the meeting of the A. A. A. S. at the University of California during the week of June 19–23, was well attended, and much enjoyed by those whose good fortune it was to be present. It brought together most of the plant physiologists of the western states, with a modest attendance from the east. The papers submitted were pooled with those of a physiological nature submitted to the Botanical Society of America and three joint half-day sessions were held. A wide variety of subjects were discussed in the thirty-four papers presented. Of especial interest was a symposium on absorption conducted by D. R. HOAGLAND, S. C. BROOKS, L. R. BLINKS, F. C. STEWARD, and PIERRE PREVOT. Several papers on absorption were read also in the regular sessions so that this subject proved to be the outstanding one at this meeting. Attendance at the physiological sessions was very good, as many as one hundred being present at times. Two excellent and interesting public addresses of interest to physiologists were delivered on Thursday afternoon by Prof. GÖTE TURESSON of the University of Lund, Sweden, and Prof. H. C. THOMPSON of Cornell University.

An interesting feature of the meetings was a series of open-house exhibits and demonstrations by various departments of the University. Of

especial interest to plant physiologists were those showing the greenhouse and laboratory equipment and the experimental work under way by the Department of Botany and the College of Agriculture. The week ended with a number of excursions to such points of interest as Stanford University, University Farm and Agricultural Laboratories at Davis, California Academy of Sciences and Golden Gate Park, Muir Woods, Yosemite Park, and Mariposa Grove.

New England Section.—The first meeting of the New England Section at Amherst on May 25–26, 1934, was decidedly successful. The attendance was close to 75, which compares favorably with the number in attendance at annual meetings of the Society as a whole. The programs consisted of sixteen papers, eight on Friday afternoon, and eight on Saturday morning. A dinner was held at Draper Hall on the campus of the Massachusetts State College, Friday evening. Dr. C. G. DEUBER, Yale University, presided at the banquet, and the two speakers were Dr. H. P. BAKER, President of Massachusetts State College, and Dr. F. A. WAUGH, Professor of Landscape Architecture. It was a very happy occasion for every one, and the meeting throughout was marked with optimistic enthusiasm. The 1935 meeting is to be held at the University of New Hampshire, Durham, New Hampshire This Section is enjoying a well-deserved success, and setting an example that members in other regions may wish to emulate.

Chemical Methods.—Only about 35 sets of the reports of the Chemical Methods Committee remain unsold. There may be among the more recently elected members some who would appreciate owning a set of these reprints with their valuable summaries of analytical technique and their literature lists. As long as they last they can be obtained from Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building, University of Wisconsin, Madison, Wisconsin. Those who want them should act quickly, before the supply is completely exhausted.

Directory.—The secretary-treasurer expects to issue a new membership directory during the coming year. Data will be required from each member in order to make it quite accurate and up-to-date. When information is requested for this directory, it is imperative that it should be furnished promptly. Dr. MURNEEK asks your hearty cooperation in getting together the material necessary for the membership list.

Endowments.—Two of the three endowment funds of the American Society of Plant Physiologists continue to increase slowly. The CHARLES REID BARNES life membership endowment fund contains nearly $1,475, and

the general endowment is now $175. The STEPHEN HALES prize award endowment fund remains at $1,100. Gifts to these endowments may be made at any time. Even small gifts are handled in such manner that they begin to yield interest toward the objectives of the endowments immediately upon receipt by the secretary-treasurer. Other scientific organizations have found that permanent endowments are necessary to their permanence and sound development. The financial arrangements of the Society contemplate a permanently growing endowment, and it is a privilege to contribute to the financial stability of its work. This privilege any member may share if he so desires.

Charles Reid Barnes Botanical Laboratory.—The botanical laboratory erected at the University of Chicago several years ago has been named the CHARLES REID BARNES Botanical Laboratory by recent action of the Board of Trustees of the University. Dr. BARNES was the first professor of plant physiology at Chicago, and was a leader in the development of experimental laboratory courses in this field. His ability as critic and lecturer, his unflinching courage and inspiring personality endeared him to all of his students and colleagues. He lost his life in February, 1910, at the age of 51 years, as the result of a fall on icy pavements as he attempted to meet an engagement with a graduate group in plant physiology. It therefore seems very appropriate that this laboratory, devoted mainly to plant physiology and plant pathology, should be named for one so intimately associated with the beginning of laboratory training in plant physiology in the United States.

Chlorophyll and Carotenoids for Research.—It is now possible to obtain chlorophyll and carotenoids for research and commercial purposes in almost any quantity desired. The American Chlorophyll Co., 3240–3244 K St. N. W., Washington, D. C., has developed extraction on a large scale, and offers chlorophyll 5 ×, which is claimed to be a highly purified preparation of chlorophylls α and β. The grade 3 × is not so pure, but a good grade. They have also prepared and can furnish salts as follows: sodium magnesium chlorophyllin, sodium iron chlorophyllin, sodium copper chlorophyllin, copper pheophytin, and iron pheophytin. The characteristic alcohol, phytol, is offered in pure and crude forms. Both carotene and xanthophyll may be had in crystalline form, and carotene in oil. One thing that should be done at once is to subject the chlorophyll preparations and the carotenoids to examination by the methods of ZSCHEILE, MILLER, and their co-workers to determine how constant are the proportions of α and β in different samples, and what these newer methods reveal as to actual purity of the preparations. The development of such sources

of material is a highly commendable project. Inquiries may be addressed to Robert H. Van Sant, President of the American Chlorophyll Co.

Plant Biochemistry.—In the July, 1933, number of PLANT PHYSIOLOGY a preliminary notice of a manual of plant biochemistry was published. The completed volume, Plant Biochemistry, By Dr. W. E. TOTTINGHAM, University of Wisconsin, has just been received from the publishers, The Burgess Publishing Co., Minneapolis, Minnesota. It is mimeographed, handsomely bound in green flexible leatherette binding, and contains 219 pages. There are 11 chapters with the following titles: General aspects; the materials of metabolism; the photosynthesis of carbohydrates; the metabolism of carbohydrates; fat metabolism; the metabolism of nitrogen compounds; physicochemical relations of the plant cell; the nature and function of enzymes; chemical aspects of respiration; salt nutrition; and climatic effects in metabolism. The work is a noteworthy attempt at orderly consideration of the chemical processes of plant life. Copies may be purchased at $4.25 each. Orders should be sent directly to the Burgess Publishing Co., 426 So. Sixth St., Minneapolis, Minnesota.

Electrokinetic Phenomena.—A very useful monograph, Electrokinetic Phenomena and their Application to Biology and Medicine, by HAROLD A. ABRAMSON, has been published by the Chemical Catalog Co., New York. The earlier chapters discuss the historical development of electrokinetics, early theories, methods of experimentation, and the more recent theories. Chapter V deals with proteins and related compounds; chapter VI with the general effects of salts on inert surfaces; chapter VII with inorganic surfaces; and chapter VIII with organic surfaces. The last three chapters consider the electrokinetics of gases, the electrical phenomena of blood cells, spermatozoa, tissues, etc.; and bacteria, antibodies, and viruses and related systems. There are several appendices, followed by author index and subject index. It is an American Chemical Society Monograph, no. 66, bound in the same style as other monographs of this series. Much of the information will be useful to plant physiologists, although the applications are most frequently made in the zoological and medical fields. The problems dealt with are of universal occurrence, and concern the materials with which any of us work. The price of the book is $7.50 per copy.

Vol. 9 No. 4

PLANT PHYSIOLOGY

OCTOBER, 1934

PENETRATION AND ACCUMULATION OF PETROLEUM SPRAY OILS IN THE LEAVES, TWIGS, AND FRUIT OF CITRUS TREES[1]

P. W. ROHRBAUGH

(WITH FOURTEEN FIGURES)

Introduction

The chief objection to the use of petroleum oils on citrus trees is the injury which may result. Various writers have noted some of the manifestations of injury, such as leaf and fruit drop, off-bloom, retardation of bloom, reduction in fruit set, failure of fruit to color properly, poor flavor, thickness of rind, softening of rind, susceptibility to breakdown and decay, and killing of twigs. Under certain conditions little understood, these injuries are rather extensive and are of considerable economic importance.

Many growers have feared that an accumulation of oil in the trees might take place from year to year, and that after a period of 10 to 15 years sufficient oil might accumulate to cause permanent injury to the health of the trees and to result in a reduction of quantity or quality of fruit.

In spite of these adverse effects, emulsions of highly refined petroleum oils are recognized as being among the best insecticides for the control of various insect pests of citrus, such as red scale, *Aonidiella aurantii* (Maskell); purple scale, *Lepidosaphes beckii* (Newman); black scale, *Saissetia oleae* (Bernard); citricola scale, *Coccus pseudomagnoliarum* (Kuwana); and red spider, *Paratetranychus citri* (McGregor).

The importance of oil spray as an insecticide is seen in the fact that during the year ending June 30, 1930, the growers in the county of Los Angeles alone spent $1,363,000 for scale insect control by means of oil sprays and fumigation (**12**). The average cost over the previous seven

[1] Paper no. 285, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

years was $1,377,000 and the average number of acres treated per year was 41,524. The acreage treated with oil has gradually been increased until during the year 1930, 55 per cent. of the total treated acreage was treated by means of oil spray. Approximately 94,000 acres of citrus orchards in California were sprayed with oil emulsions during 1931 (**2**).

KNIGHT, CHAMBERLIN, and SAMUELS (**11**) have investigated the penetration and location of spray oils in citrus trees, but their work on penetration was limited to the use of undiluted oils. A study of the penetration and accumulation of oil applied as emulsions seems very desirable, as this is the form in which the oil is used in the commercial orchard.

The present paper contains data relating to (1) the entrance of the oil into the leaves and twigs, (2) the movement of the oil in the tree, (3) the tissues in which the oil is finally deposited, (4) the rate of disappearance of oil from the tissues, and (5) the anatomical effects which result from the use of spray oils.

Histological studies

OILS USED AND THEIR SPECIFICATIONS

The spray oils and oil emulsions used in most of these experiments are materials commonly used in the commercial spraying of citrus trees in California. The specifications of the oils are given in table I.

TABLE I

SPECIFICATIONS OF OILS USED IN THESE EXPERIMENTS

OILS	VISCOSITY	UNSULPHONATABLE RESIDUE
		%
Oronite technical	100–105	99
Oronite X (a special oil)	100	85
Oronite cosmetic	70	99
Oronite mineral seal	50	94
Special 80	80	85
Volck concentrate	100–105	99
Volck light	50	94
Avon heavy	95	94

The "tank-mix" emulsions referred to in this paper are emulsions made by placing the emulsifier in the spray tank with the water. When the water and emulsifier are mixed and the latter is dissolved, the undiluted oil is added. The spray machine mixes these until an emulsion is formed.

In these experiments the percentage of oil used refers to the percentage of actual oil in the completed emulsion; that is, when Volck Concentrate (a

commercially prepared emulsion) is used, enough more of the heavy prepared emulsion is added to make up for the 18 per cent. of water already in the heavy emulsion.

METHODS

Two methods of investigation have been followed in these experiments. One method consists of a histological study of the penetration, location, and accumulation of the oil in the tissues of the plant. The other method consists of an extraction and quantitative measurement of the oil found in a given quantity of plant tissue.

TECHNIQUE

There have heretofore been no staining methods which satisfactorily distinguish between the natural plant oils and the petroleum spray oils in the plant. It is difficult for anyone using the methods of KNIGHT, CHAMBERLIN, and SAMUELS (**11**) to be certain which oils are plant oils and which are spray oils, since they both stain red. This is especially true of sections of the stems.

The staining methods described below have proved very useful for distinguishing between the natural oil and the petroleum spray oil in sections of citrus tissue.

The technique found most practical for the histological studies consists of cutting sections of fresh tissue from the part of the plant to be investigated. This is best done with a sliding microtome having a freezing attachment. The sections are then placed in a normal solution of potassium hydroxide or Bouin's killing solution for 20 minutes, or they may be placed directly in a dye solution made up as follows: To a saturated aqueous solution of nile blue sulphate, 0.5 per cent. of sulphuric acid is added and the mixture is boiled under a reflux condenser for 4 or 5 hours. (This solution should be made as nearly alkaline as possible without a change of color; if too much hydroxide is added the solution turns a bright red color.) A solution of 50 per cent. alcohol and 50 per cent. acetone is then saturated with oil red O dye. One part of the prepared nile blue sulphate solution is then added to 2 parts of the oil red O solution. This is allowed to settle overnight and is then filtered, after which it is ready for use.

The nile blue sulphate solution is SMITH and MAIR'S (**16**) stain for fat. It is supposed to stain fatty acids blue and to stain the neutral esters red. Most of the natural oils of citrus stain blue with this dye, but the petroleum oil stains very little or not at all. The combination of the two dyes as just described, however, gives a brown or black color to the plastids and most of the natural oils. The petroleum spray oil, the oil in the oil glands, and frequently a small quantity of plant oil or resin which lies between

the cells of a narrow band in the phloem region of twigs, give a bright red color with this dye mixture unless the sections have been previously placed in normal potassium hydroxide solution or Bouin's killing solution. If previously placed in potassium hydroxide or Bouin's solution, only the petroleum oil shows the red color. Leaf sections stain satisfactorily in one hour while stem sections require several hours, but both are better and more permanent if stained overnight. Best results have been obtained without destaining. If the dye becomes too concentrated, owing to evaporation of alcohol and acetone, large round globules of dye appear on the sections. This may be avoided by replenishing the alcohol and acetone. When stained sufficiently, the sections are rinsed in water and then mounted in glycerin jelly.

The oil red O dissolved in pyridine as recommended by PROESCHER (**15**) may also be used either with or without the nile blue sulphate. Sections stained with oil red O in pyridine show the oil more in the form of large globules than as a film between the cells of the tissue, as shown in the sections stained with the acetone-alcohol solution. The use of alcohol and acetone has a more dehydrating effect on the tissues than has the use of pyridine.

It was found desirable to avoid all rinsings and washings of the sections which were not absolutely necessary, since the oil is mostly in the form of a film between the cells of the plant tissues and the more the sections are washed or the longer they are left in any solution, the more tendency there is for the oil to become globular and to come out of the tissues into the liquid. This is especially true when the sections are in water solutions. If sections of freshly sprayed tissues are cut and placed in water for a few hours, small droplets of oil will emerge and may be found on the surface of the water. The dyes, when used according to this method, have a tendency to fade slowly and to diffuse out into the mounting medium, so that after a week or two the sharper contrasts are lost. It is possible that some other mounting medium might prove more satisfactory in this respect. Glycerin jelly is the only medium which has been tried.

Another method of staining which has proved to be of interest and which may be used to some extent in this type of work is the nascent indophenol blue method as used by DUFRENOY (**6**). This method of staining is as follows: Dissolve 1.5 gm. of thymol crystals in 100 cc. of warm water on a water bath. Add 0.5 cc. of 33 per cent. potassium hydroxide. (This solution will keep for several weeks.) Next dissolve about 0.5 gm. of p-aminodimethylanilinehydrochloride in 50 cc. of water. This makes a very light rose-colored solution. This is decolorized by adding, drop by drop, a solution of potassium hydroxide until the rose color disappears. Avoid adding too much potassium hydroxide. Add 50 cc. of the thymol

solution. Place sections of material to be stained in this solution. After a few minutes the blue, lipoid-soluble, nascent-indophenol blue appears in the solution.

This dye stains the natural oils, lipoids, and oleo resins a bright blue and the petroleum oil a reddish blue or violet. It can be used only for temporary mounts, as it lasts only about half an hour. The larger globules of petroleum oil are readily distinguished by this method, but since the oil does not stain deeply, it is often impossible to distinguish between the films of oil and the air in the intercellular spaces. This dye has the advantage of being in a water medium and there is therefore no dehydration of the section. It probably allows one to view the larger oil globules more nearly as they are in the unsectioned leaf than is possible in sections mounted in a dehydrating medium. Figure 3*C* is a section of the upper side of an orange leaf stained by this method, showing oil between the palisade cells.

Natural oils in the plant

The first histological observations were made on sections of tissues from healthy, unsprayed citrus trees of different ages. This was done to determine whether natural plant oils were present, and, if present, to determine their location and prevalence. Many small droplets of natural oil were found in the cells of the cortex, in the medullary ray cells of the stem, and in the chlorophyll-bearing cells of the mature leaves. Figure 1, *A* and *B*,

Fig. 1. Natural oil in cells of medullary ray: *A*, tangential section of unsprayed orange twig; *B*, radial section of unsprayed orange twig.

shows the natural oil in the medullary ray cells. When the leaves are very young and immature, the oil is either lacking or it is to be found only in small quantities. There is a wide variation in the quantity of oil present in different trees and in different parts of the same tree.

Entrance and location of spray oils in the plant

Leaves.—The upper surface of mature orange leaves was painted with undiluted Oronite Technical oil. The leaves were kept for the next 24 hours at a temperature ranging between 75° and 85° F. An examination at the end of this time showed that only occasionally a small amount of oil had penetrated through the leaf to the lower epidermis. The upper surface of a citrus leaf has no stomata. At a temperature of 95° F. or above, the oil sometimes passed through to the lower surface of the leaf at the end of the 24-hour period. The cuticle was rather slowly penetrated by the oil which then passed between the cells of the epidermis and the palisade layers into the spongy parenchyma (fig. 2, *A* and *B*). The final depth to which

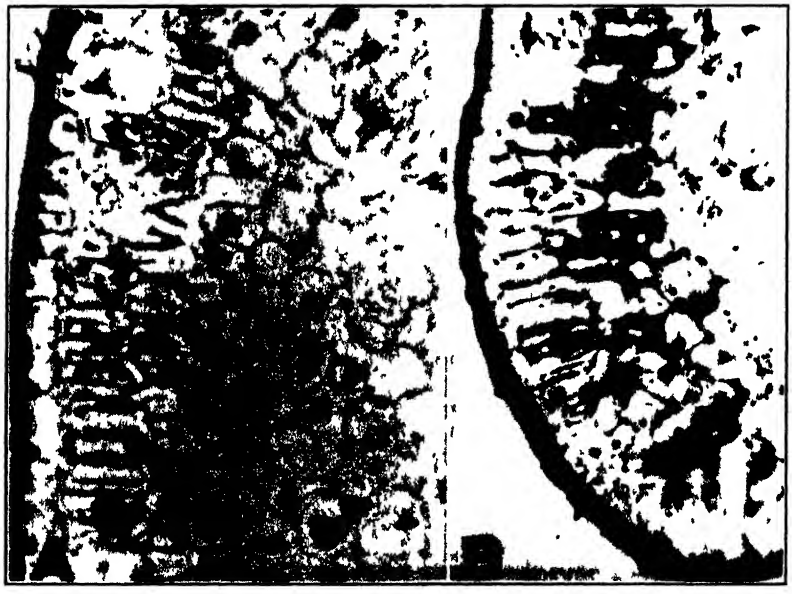


Fig. 2. Cross-section of orange leaf showing spray oil between cells of palisade region: *A*, near margin; *B*, at margin.

it penetrates depends not only on temperature but also on the quantity of oil applied. The movement of the oil appears to be due entirely to capillarity.

Oil painted on the lower surface of leaves showed a quicker penetration.

The indications were that the oil entered largely through the stomata. At first it spread in fan-like areas from the stomata (fig. 3 *A*). It may also penetrate the cuticle to some extent.

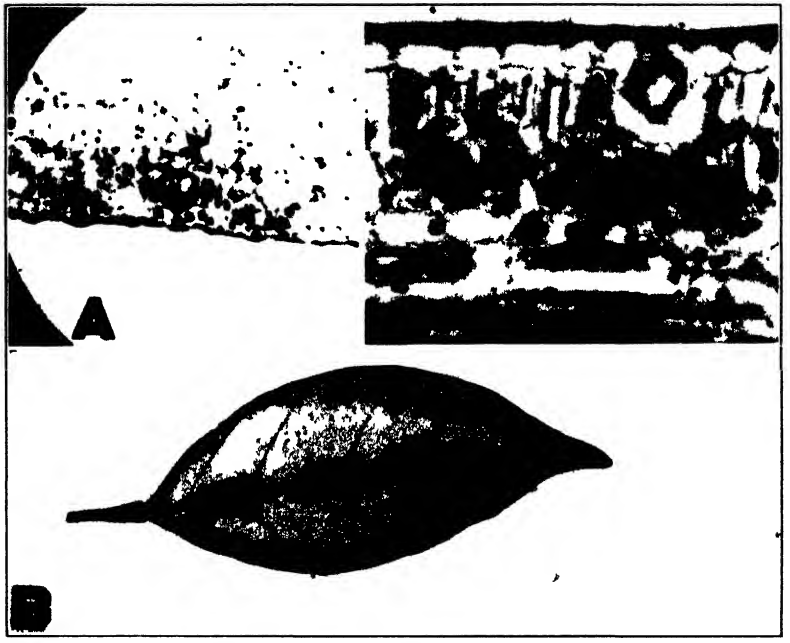


FIG. 3. *A*, section of lower surface of orange leaf showing spray oil which has entered leaf through stomata; *B*, orange leaf showing oil-soaked areas caused by oil-spray emulsion; *C*, section of leaf showing spray oil between palisade cells stained with indophenol blue. (Note rounded globules as compared with those of figure 2, *A* and *B*.) Leaf sprayed with 2 per cent. Avon Heavy.

Sections of leaves previously sprayed with oil and water emulsions showed that the oil in this form penetrated in a manner similar to that of undiluted oil, the only visible difference being in the quantity of the oil that penetrated.

When only a small quantity of oil was applied, or when an excessive amount of spreader was used, there was much less penetration of the upper epidermis and often only a small amount of oil penetrated the lower surface of the leaf. Sections of leaves which were sprayed with 2 per cent. Oronite Technical oil, using eight times the normal quantity of spreader in a tank-mix emulsion, showed a light penetration of oil which was well distributed on the lower surface of the leaves. There were only small oil-soaked areas on the margins of these leaves, and sections showed only a small quantity of oil at the margins and but little oil at any other place in the leaf.

These results indicated that under some conditions the oil may scarcely penetrate the upper surface at all. Under the conditions of ordinary commercial spray practice the oil usually concentrates along the veins, midrib, and especially the leaf margin, and there penetrates, producing an oil-soaked appearance. Figure 3 *B* is a drawing of a leaf showing an oil-soaked area, which is due to the presence of oil between the palisade cells. This oil-soaked area, when resulting from the application of the heavier oils, can be detected throughout the life of the leaf, which is sometimes more than two years. It is, however, often necessary to allow the leaf to wilt slightly before the darkened area becomes evident. The extent of the oil-soaked areas of the leaves may be used at least to some extent as an indicator of the quantity of oil in the leaves.

TWIGS.—In a preliminary test, about 8 cm. of an orange twig 1.5 cm. in diameter was painted with Oronite Technical oil which had been saturated with oil red O. One week later the twig was cut off and sectioned. The sections showed that the oil had penetrated to the pith and had spread

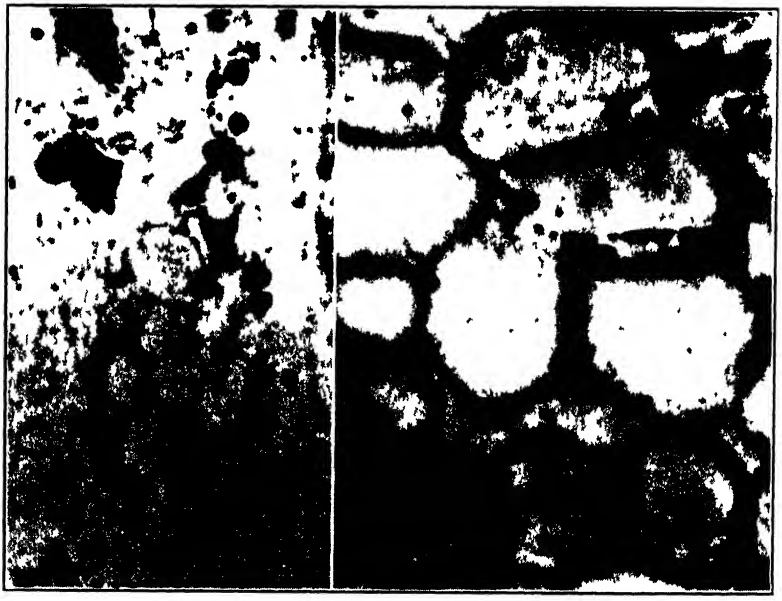


FIG. 4. Spray oil between cells of cortex of orange twig: *A*, longitudinal section; *B*, cross-section.

on the surface about 2 cm. in each direction beyond the place of application. Longitudinal sections of the stem showed that the oil had penetrated lengthwise of the stem on the inside to about the same extent that it had spread on the surface.

Other stems of various sizes were painted with dyed Oronite Technical and also with Mineral Seal oil with similar results. Twigs which had smooth bark absorbed the oil more slowly than those with split, rough bark. Cross-sections of twigs with split, rough bark showed streaks of red beneath these splits to, or nearly to, the centers of the twigs.

Sections of twigs sprayed with ordinary emulsions of Oronite Technical or Mineral Seal oil showed the same type of penetration, but to a much less extent. When the emulsions were used, the oil seldom penetrated to the cambium, although it could be found between the cells of the outer one-third of the cortex. Figure 4, *A* and *B*, shows the presence of spray oil between the cells of the cortex. Figure 5 *A* shows a section through the cortex of a normal unsprayed twig with cambium at one side and epidermis

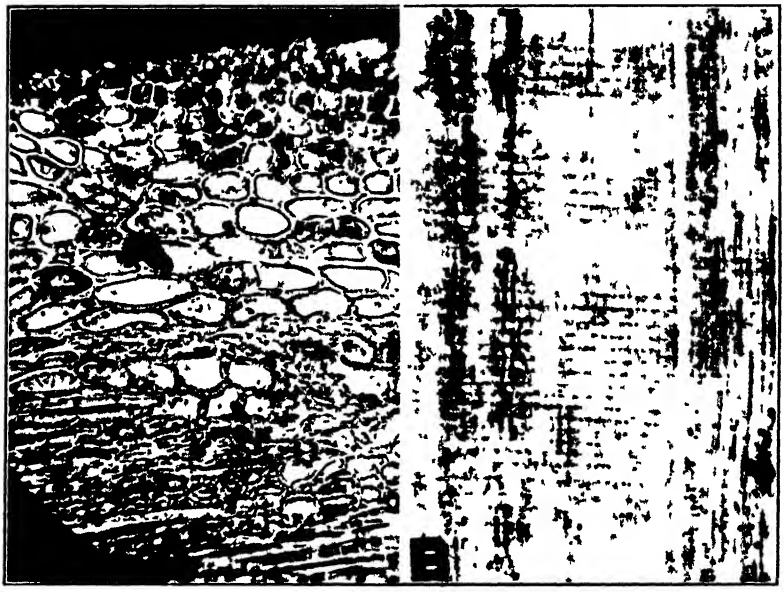


FIG. 5. *A*, longitudinal section of orange twig showing structure of cortex; *B*, radial section of orange twig showing spray oil between cells of medullary ray and in wood fibers underneath ray.

at the other. Under ordinary commercial conditions the oil would be limited to the outer one-third of this section.

Lemon, grapefruit, and orange trees were sprayed in September, 1928, with undiluted oils of various viscosities and of various percentages of sulphonatable or unsaturated hydrocarbons. Two years later, observations were made on this material. Oils of viscosity below 70 were very seldom found in these tissues and then in only very small amounts. Twigs of the trees sprayed with oil above 70 seconds' viscosity showed a distinct ring of

oil present in the lumen of the wood cells at the place where the cambium was at the time the spray was applied (fig. 6 *A*). There were also a few scattered deposits of oil deeper in the twigs. These decreased in size and frequency toward the center of the twigs although sometimes oil could be found just outside the pith of the smaller twigs in connection with gum

FIG. 6. Cross-section of lemon twig 2 years after spraying with pure oil, showing heavy band of oil in cells of woody cylinder: *A*, highly magnified; *B*, less highly magnified, also showing oil near pith.

deposits (fig. 6 *B*). No oil could be found outside the place where the cambium was at the time of the spray application. No such condition has been found following the application of commercial emulsions as usually used for insect control.

The quantity of spray oil which remains in a twig depends largely upon its volatility.

When undiluted oils or excessive quantities of oil emulsions are put on citrus twigs, the path of the oil which enters the twigs appears to be largely through splits if there are any in the outer bark. It then passes between the cells of the epidermis or lenticels and between the cells of the cortex until it gets to the medullary rays. It follows along the sides of the ray cells (fig. 7, *A* and *B*) past the cambium and into the woody cylinder. There it often enters and follows the lumen of the wood fibers or lubriform cells (fig. 8 *A*) lengthwise for some distance, often filling many of these

cells with the oil as it goes. Figure 8 *B* shows a section of a small twig containing spray oil between the cells just at the edge of the pith.

In contrast to this, sections of twigs sprayed with various commercial emulsions containing 2 per cent. oil or less show that the oil did not ordinarily penetrate to the cambium. Some sections of lemon twigs which had been sprayed with 3 per cent. Oronite Technical Mixol showed that a trace of oil had gone into the wood fibers. Sections of orange twigs which had been sprayed with 4 per cent. oil of 100 seconds' viscosity put on as a tank-mix emulsion also showed some oil in the wood.

FRUIT.—Sections were made of oranges from both navel and Valencia trees which had been sprayed two months previously with 2 per cent.

FIG. 7. Radial section of orange twig 48 hours after painting with Oronite Technical oil, showing spray oil between cells of medullary ray: *A*, faint lines of oil between cells of older portion of ray; *B*, oil between cells of new portion of ray.

tank-mix emulsions of Oronite Technical and Mineral Seal oils, and 2 per cent. emulsions of Volck Concentrate and Volck Light oils. Sections of the fruit where the Oronite Technical and the Volck Concentrate oil were used showed some oil penetration. These oils were found only in the outer 1 mm. of the rind. There are numerous oil glands in the outer portion of the rind, and the spray oil was found most abundantly between the cells which lie between these oil glands. The oil was not found evenly over the fruit but occurred only in spots and sometimes on only one side of a fruit. Sections of orange sprayed with the lighter oils, Mineral Seal and Volck Light, showed almost no spray oil present in the fruit. It was only occasionally that one could find a trace of oil.

Sections of lemons sprayed with Volck Concentrate and with Volck Light oil one month previously showed only a small amount of penetration

around the buttons of those fruits sprayed with Volck Concentrate, and no evidence of oil penetration in those sprayed with Volck Light oil.

Figure 9 *A* shows spray oil between the cells of orange rind. Figure 9 *B* is a section of lemon rind showing spray oil between the cells after the lemon had come from the packing house. This fruit showed considerable browning of the albedo on one side but the oil was also prevalent on the opposite side of the fruit.

FIG. 8. Longitudinal section of orange twig 2 years after spraying with 4 per cent. emulsion of Volck Concentrate: *A*, oil in wood fibers near large xylem tubes; *B*, spray oil between cells near pith of small stem.

ROOTS.—Sections of the roots of trees which had been sprayed with undiluted oils or with emulsions have not shown any evidence of oil in them except in the case of some small trees in pots which were sprayed with undiluted oil, and the oil had run down the stem. In these plants oil was found in sections of roots taken from about 2 inches below the surface of the soil.

MOVEMENT OF SPRAY OIL IN CONDUCTING SYSTEM OF PLANT

KNIGHT, CHAMBERLIN, and SAMUELS (11) referred to the conduction of oil by the plant. Some investigators of petroleum spray oils have taken

for granted that since the oil penetrated the leaf, it must be conducted away from it into the plant stems.

To find how far the oil was conducted, twigs bearing twenty to thirty mature leaves were selected on large healthy orange trees. The twigs were used in four groups of three each. Using 2 per cent. emulsions, one group was sprayed with Volck Concentrate, another with Volck Light, a third with Oronite Technical, and a fourth with undiluted Mineral Seal oil. One twig from each group was sectioned after 24 hours, another after 3 days, and the other after 10 days, to see how far the oil had gone down the stem.

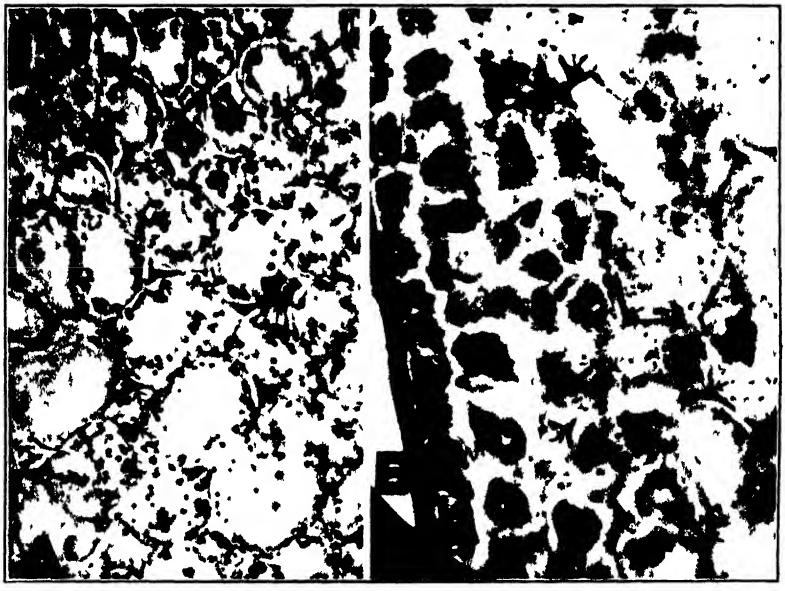


FIG. 9. *A*, section of orange rind showing spray oil 10 days after spraying with Oronite Technical oil; *B*, section of rind from lemon which had shown considerable browning of albedo. Oil was found on side not showing browning of albedo as well as on browned side. This lemon had been through coloring process of packing house.

In no case was there any indication of oil conduction from the leaves into the twig. In some instances the oil had apparently gone 1 or 2 cm. along the stem by capillarity, but no oil which could be identified as spray oil was found in the conductive tissues in any case.

This experiment was repeated, using oils dyed with oil red O, but again no evidences of oil conduction were found.

HISTOLOGICAL INDICATIONS OF INJURY

Sections were made from orange and lemon trees which had been sprayed 2 years previously with undiluted oils varying in viscosity from

30 to 288 seconds and varying in unsulphonatable residue from 98 to 50 per cent.

Those sprayed with oils containing from 70 to 50 per cent. of unsulphonatable residue showed severe injury in the form of breakdown of the cells in the region of the cambium, leaving a ring of large cavities at least part of the way around the twig (fig. 10, *A* and *B*). These cavities were filled with gum and often tyloses extended out into them from the surrounding cells. The new wood which had been laid down after the spray had been applied was wavy and knurled in appearance.

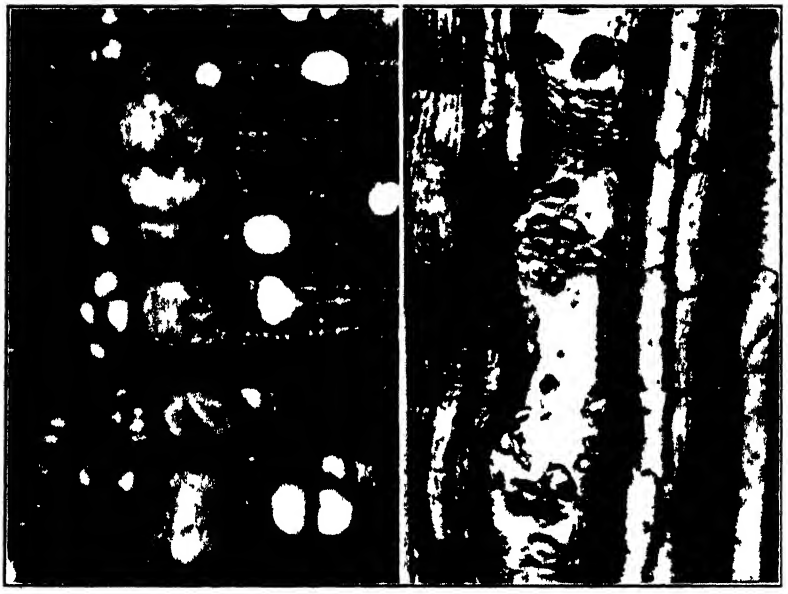


FIG. 10. Breakdown of cells caused by unsulphonatable oils: *A*, cross section of lemon twig; *B*, longitudinal section of lemon stem.

Sections of stems from trees sprayed with oils of more than 70 per cent. unsulphonatable residues did not show these cavities, but practically all showed some irregular or abnormal cells in that region which had been cambium or primary xylem at the time the spray was applied. These cells appeared wavy or corrugated in longitudinal sections. The walls of the wood fibers in this region were usually thinner than those of normal ones.

Sections of twigs of trees sprayed with undiluted 100-viscosity oil containing 85 per cent. of unsulphonatable residue showed a pronounced wavy appearance. They also contained considerable oil in the wood fibers. No histological indications of injury have been observed when commercial emulsions have been used.

PETROLEUM OIL WITHIN THE CELLS OF THE PLANT

Petroleum oil has not been identified in the living cells of the plant such as the chlorophyll-bearing cells of the leaf and the cells of the cortex, phloem, and medullary rays of the stem, although it sometimes appears that an occasional cell of the living part of the leaf or stem has petroleum oil in it. Cells containing large oxalate crystals frequently contain spray oil. If a freezing microtome is used there is much less evidence of oil in any living cells.

The oil enters the wood fibers and often completely fills many of them, but this is only in stems which have been sprayed or painted with undiluted oil or whenever enough oil has been applied so that it penetrates the woody cylinder.

There are also evidences that at times the oil gets into the xylem tubes, but this happens only when the stem is practically saturated with oil. Oil has never been found in the xylem tubes of commercially sprayed trees.

PENETRATION OF SPRAY EMULSION, AS SUCH, INTO LEAF AND STEM

KELLY (**10**) has indicated that the spray emulsion enters the leaf of the apple. There is no indication that this is true with citrus leaves. If citrus leaves are placed in water under reduced pressure, the air is removed, and when the pressure is again brought to normal the leaf fills with water. This is readily noticeable by the water-soaked appearance of the leaf. Such appearances do not normally occur in leaves sprayed with oil emulsions. The oil-soaked areas mentioned in this paper resemble to some extent the water soaking, except that the oil soaking occurs along the midrib and along the margin, and when the heavier oils are used it is permanent throughout the life of the leaf.

To determine more fully whether water would enter the leaves of citrus, orange twigs including the leaves were sprayed with solutions of different water-soluble dyes. After the leaves had dried, the twigs were cut and taken to the laboratory. Sections of the leaves and twigs were made but no traces of the dyes could be found. The dyes used were acid fuchsin, trypan blue, thionin, malachite green, and safranin O. In these tests no emulsifier was used. It was omitted because the absence of an emulsifier usually increases the oil-soaked appearance when applying oil sprays.

In sections of leaves which had been sprayed with emulsions, whether tight or quick-breaking emulsions, the oil showed the same relative distribution through the leaf as when undiluted oils were applied.

If, as KELLY (**10**) has stated, the oil enters the plant in the form of an emulsion, the globules should be found scattered throughout the mesophyll and other tissues having intercellular spaces. It is certain that the oil could

get no farther than into the intercellular spaces of the leaf as an emulsion, for it would have to pass through a cell wall in order to get into the conduction tract of the plant. In passing through a cell wall it would have to go through as a solution or as a much finer emulsion than is common in these sprays, at least as long as the cell was alive. There seems to be no significant difference in the appearance of the oil which has entered through the upper surface where there are no stomata and that which enters through the stomata of the lower surface of the leaf, except that the oil is more in films than in globules, because of the fact that the cells of the palisade layers are much closer together. As long as the oil is in the palisade region it has little of the globular appearance. When sufficient oil is added so that it goes beyond the palisade layers into the spongy parenchyma, it takes on the same globular appearance that it has when it enters from the lower side through the stomata. This is probably due to the fact that it has more room to spread out and form globules.

While observations point to the conclusion that the oil enters the plant almost entirely as a continuous oil film drawn in by capillarity, it is difficult to say with any certainty that the force which pulls the oil into the plant tissue is capillarity. If the walls of the cells in the leaf and in the stem are wet on the outer side, it is difficult to explain this movement by capillarity, since capillarity depends upon the cohesion of the liquid to the surface of the capillaries. If the surface of the cell walls is covered with a lipoid film, or some other surface to which the oil could cohere, then capillarity might well be the explanation of the movement of the oil into the tissue.

The section shown in figure 3 *C*, which was stained with dye in a water medium, shows clearly that the masses of oil are rounded on the ends and do not appear to be adhering to the cell walls. This, however, may be due to the water which has been added to the section. There is nothing to indicate that the intercellular spaces are filled with water emulsion by spraying. They do not have the appearance of being water-soaked, except perhaps along the outer edge and along the midrib. This water-soaked appearance is evidently not due to water, since it stays for at least some weeks and sometimes years; whereas if it were due to water it would disappear rapidly. Sections of the leaves indicate that this water-soaked appearance is due to oil between the cells of the palisade layers.

Physical effect of oil around the cells of the plant

The question arises as to whether some of the cells of the plant, especially in the oil-soaked area of the leaf, are completely surrounded by oil. Cross-sections of leaves through the oil-soaked parts of the palisade layers and sections cut parallel to the dorsal surface through these areas appeared

as though some of the palisade cells were completely surrounded. It is certain that if these cells are not completely surrounded they at least have their exposed areas greatly reduced. If these cells are completely surrounded they must have some protoplasmic connections with those cells which are not surrounded, or there must be an adequate interchange of mineral solutes and gases through the oil film, for there is no evidence of any dead cells. However this may be, at least a small number of citrus leaves remain on the trees for as long as two or more years after being sprayed, without showing any ill effects from the oil.

In this connection it is recognized that an oil layer many times the thickness of the films (which are usually not more and generally are much less than 4 μ thick) around these cells is necessary in order to produce anaerobic conditions. It is also recognized that oils should not always be considered toxic to all kinds of protoplasm. DUGGAR (**7**), MELHUS and DURRELL (**14**), and GOLDSWORTHY and SMITH (**9**) have shown that petroleum products stimulate the growth of some fungus spores. CONN (**3**) has shown that white mineral oil, vaseline, and paraffin produce a marked stimulation of the growth of *Streptococcus*. CONTOLEON and JOANNIDES (**4**) found no demonstrable effect of liquid petrolatum in retarding bacterial growth *in vitro* or in the intestines. BAILEY (**1**) has kept cambium initial cells alive and actively streaming for 500 hours (about 21 days) in White Russian oil. MCWHORTER (**13**), however, has reported that Volck Concentrate is a good control for mildew on roses.

Quantitative determination of petroleum spray oils in citrus leaves and twigs

Several investigators have worked on quantitative methods for measuring the spray oil retained by leaves and other surfaces. SMITH (**17**) has described methods for weighing the oil deposited on glass slides and on leaves by various oil emulsions. ENGLISH (**8**) has described a method for measuring the oil retained by citrus leaves. This method consists of cutting fifty discs, each 10 sq. cm. in area, from sprayed leaves and extracting them by shaking them twice for 1 minute, using 50 cc. of ethyl ether each time. ENGLISH'S method has been tried out in this laboratory, and after shaking the leaves with ether for as long as 30 minutes, it was found that not all of the petroleum oil was removed from them. This was determined by drying the leaves and then extracting them again with petroleum ether and determining the oil content as described in this paper. ENGLISH'S method, however, may give results which are relative to the actual quantity of oil present in the leaf.

It is shown in figure 3 *B* of this paper that the oil is not evenly distributed throughout the leaf, but is concentrated along the midrib and espe-

cially near the margin. It is therefore believed that the use of the discs from the leaves does not give a representative sample of the oil deposit.

DAWSEY and HAAS (5) have recently described another method for measuring the quantity of petroleum spray oil deposited on camphor, pecan, and orange leaves. This method of extracting the oil from the camphor and pecan leaves is similar to the method used by ENGLISH (8). These investigators also experienced some difficulty in extracting all of the oil from citrus leaves, and they have somewhat modified ENGLISH'S method.

PREPARATION OF MATERIAL

Two methods were used in the selection of samples of leaves for extraction. In most of these tests the samples were obtained by picking a given number of leaves from each of several trees (usually three to five trees were used), and usually not fewer than 500 leaves were used for the total sample. A definite number of leaves was selected from each of the four sides of a tree. Only healthy, medium-sized leaves were collected. As soon as the samples of leaves were picked they were placed in paper bags and taken to the laboratory where they were dried. Two methods of drying were used. The samples used in the tests recorded in table VI and in figures 11 and 12 were placed in an oven to dry at a temperature not to exceed 80° C. In all other data given, the samples were dried in wire trays at room temperature. As soon as the samples were dry they were ground to powder in a Wiley mill, using a screen having 1-mm. holes. This ground material was then placed in tight tin containers until such time as the extractions could be made.

In collecting samples from commercially sprayed groves a different method was used. The samples were obtained by collecting two branches from opposite sides of each of a number of trees. These branches were then tied together, the bundle tagged, and taken to the laboratory where the leaves were picked, dried, and ground. If the trees from which these samples were collected had put out new leaves which had grown to maturity so that they could not be distinguished from the sprayed leaves, the twigs were left until the leaves wilted and then only those leaves which showed some oil-soaked area were picked.

Samples of twigs were obtained in the same manner as were the leaves from commercial groves. After these twigs had been brought to the laboratory and the leaves picked off, the twigs under 0.25 inch in diameter were cut in pieces about 6 inches long. They were then ground in the Wiley mill and placed in tin containers.

SOLVENTS USED IN EXTRACTING OIL

Acetone, benzene, ethyl ether, and petroleum ether were tried as solvents. Since the petroleum oil must be separated from the oily or waxy

materials extracted from the plant tissues, it is desirable to have an extract that is as nearly free as possible of the latter materials. Petroleum ether is a good solvent for the petroleum oils, and since it extracted much less of the plant materials than did the other solvents, it proved to be satisfactory for this work. There are probably no two lots of petroleum ether which will be found alike even though they may be labeled b.p. 30° to 60° C. Some lots have been obtained which are much less volatile. It was because of this that it was found necessary to run a stream of air into the flasks as described later. The ether obtained from the Central Scientific Company of Chicago has given satisfactory results, while ether obtained from one other source has not been suitable for this work.

Methods of extracting and measuring spray oil

Twenty-five gm. of each of four samples of ground leaves or twigs were weighed out and placed in four Erlenmeyer flasks of 300 cc. capacity. About 175 cc. of petroleum ether (boiling point 30° to 60° C.) were added to each flask. The flasks were placed on the table for 30 minutes and shaken at intervals of 5 minutes during this time. The extract was filtered from the plant tissue by using a specially made Buchner funnel. The sides of the cup part of this funnel are 12 cm. high and the inside diameter of the cup is 9 cm. The contents of the extraction flask can be emptied into this funnel rapidly with little danger of loss. The funnel was placed on a suction flask and the solvent and extract were drawn through (not too rapidly) by means of reduced pressure. The plant material was then washed several times with petroleum ether, these washings being added to the extract. The original filtrate was then returned to the original flask. The four flasks of total filtrate were placed in a constant temperature water bath at 60° to 65° C. and each connected to a condenser for recovering the ether as it distilled off. When the ether had distilled down to about 5 cc. or less, the flasks were disconnected from the condenser and a stream of air from the compressed air line was run into the flasks by means of a small glass tube inserted into the flasks. The stream of air was allowed to run slowly against the bottom of the flasks for approximately 10 minutes, or longer if there was a large quantity of plant extract present. The air aided greatly in driving off the last traces of ether.

When all of the ether had been driven off, 5 cc. of sulphuric acid of specific gravity 1.840 to 1.835 were added to each flask. The flasks were then allowed to remain in the water bath for 5 minutes, after which 5 to 10 cc. more of acid were added. The flasks were rolled around so that the acid came in contact with any material on the sides of the flasks. They were then returned to the water bath for another 5 to 10 minutes. Best results were obtained by avoiding any hard shaking or beating, as this

tended to break the oil into very fine globules, making it difficult to bring the oil to the surface of the acid when centrifuged.

As soon as all plant material was digested, and when no black undigested material was left adhering to the bottom or sides of the flasks, the contents were transferred to Babcock milk test bottles by means of a small glass funnel, the stem of which had been drawn out to a 2-mm. opening. The flasks were then rinsed several times with 5 to 10-cc. portions of acid until the test bottles were filled. The bottles were filled only to the bottom of the graduated column, as expansion due to heating in the centrifuge ran the bottles over if they were filled too full.

When all four samples were in the test bottles they were placed in a high-speed centrifuge which was equipped with a thermostatically controlled heating unit, keeping the temperature between 60° and 70° C. This heating unit was composed of a 1000-watt heating coil from an electric stove. It was fastened to the under side of the centrifuge lid. A De Khotinsky thermoregulator was inserted through a hole drilled in the top of the centrifuge. This heater raised the temperature in the centrifuge to 65° C. in about 10 minutes and the temperature seldom varied more than 2°. The speed of the centrifuge was about 1200–1300 r.p.m. Each set of samples was centrifuged for not less than 30 minutes after the centrifuge had become heated. After centrifuging, the test bottles were removed and the length of the oil column in each was measured.

In most of the work reported here, Babcock bottles which had the same length of graduated neck were used. The graduations were not used, but a vernier caliper was used to measure the length of the oil column. The readings were recorded in millimeters and later converted to cubic centimeters. The graduated necks of the Babcock milk test bottles hold 1.58 cc. and are divided into 80 divisions; thus each division has a capacity of 0.0197 cc. The number of these divisions that are filled with oil after centrifuging when multiplied by the factor 0.0197 gives the number of cubic centimeters of oil in a given sample. For example: If a 25-gm. sample of plant material yielded 12.5 divisions of oil, then $12.5 \times 0.0197 = 0.246$ cc. of oil per 25 gm. of plant material, or 0.984 cc. per 100 gm. of plant material. This method of measuring the oil in the Babcock bottles may be found more satisfactory than measuring the oil column with the vernier caliper.

The data given in table II are the results of tests to determine whether an appreciable loss of the lighter oils would result from the treatment given them in the extraction process. These data indicate that about 2 per cent. of the lighter oils (such as Mineral Seal) will be lost when heated on a water bath at 65° C. for 70 minutes. Mineral Seal oil with a viscosity of 50 was used.

The volatile portion of such oils probably disappears during the dry-

TABLE II

RATE OF DISTILLATION OF PETROLEUM ETHER FROM MINERAL SEAL OIL AND AMOUNT OF LOSS OF OIL DURING EVAPORATION OF ETHER

TEST	WT. OF OIL IN FLASK	WT. AFTER BEING AT ROOM TEMPERATURE			WT. AFTER BEING IN WATER BATH 70 MIN. AT 65° C.	LOSS
		5 HR.	24 HR.	46 HR.		
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.641	1.641	1.637	1.637	1.607	0.034
2	1.644	1.647	1.644	1.642	1.608	0.036

ing of the test samples; therefore the oil obtained in the extraction of dried leaves is probably not entirely accurate. The loss of oil from drying the leaves is probably very small from samples of leaves collected more than 10 days after the spray has been applied, because the more volatile portion of the oil has already volatilized while the leaves were on the tree.

In order to determine the length of time necessary to drive all of the ether from the flasks, samples of dry, ground leaf tissue weighing 29 gm. each were extracted. This material had not been sprayed and therefore it had no spray oil in it. The ether was then driven off in the manner already described. When the material had come to a constant weight, approximately 0.2 cc. of Mineral Seal oil was pipetted into the extract in each flask. The flasks and contents were again weighed. Ether was added and the

TABLE III

RATE OF DISTILLATION OF PETROLEUM ETHER FROM MIXTURES OF PLANT EXTRACT AND MINERAL SEAL OIL

SAMPLE NO.	WT. OF PLANT EXTRACT	WT. OF OIL ADDED	HEATED AT 65° C.				
			10 MIN.	20 MIN.	30 MIN.	40 MIN.	50 MIN.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.263	0.160	+ 0.435	+ 0.181	+ 0.014	− 0.006	− 0.009
2	0.472	0.188	+ 0.494	+ 0.185	+ 0.023	− 0.005	− 0.008
3	0.292	0.194	+ 0.509	+ 0.181	+ 0.043	− 0.005	− 0.007
4	0.294	0.184	+ 0.469	+ 0.183	+ 0.033	− 0.005	− 0.007

contents of the flasks dissolved. The flasks were again placed in the water bath and the ether distilled off at 65° C. During this process the flasks were taken out and weighed at each 10-minute interval. The weight of

extract, the weight of oil added, and the weight above or below the original weight before the ether was added, are given in table III.

At the end of 40 minutes all the flasks showed a very small loss in weight, indicating that all the ether had distilled over by that time.

TABLE IV

COMPARISON OF DISTILLATION RATE OF PETROLEUM ETHER AND ETHYL ETHER FROM PETROLEUM OIL

SOLVENT	WT. OF OIL ADDED	WEIGHT AFTER HEATING		
		10 MIN.	20 MIN.	30 MIN.
	gm.	*gm.*	*gm.*	*gm.*
P. E.	0.176	+ 0.439	+ 0.205	+ 0.005
P. E.	0.169	+ 0.452	+ 0.151	− 0.001
E. E.	0.172	+ 0.471	+ 0.137	− 0.000
E. E.	0.177	+ 0.485	+ 0.124	− 0.003

In order to determine whether it was more difficult to free the oil from petroleum ether than from ethyl ether, a similar test was made but without the leaf extract, using two samples with petroleum ether and two others with ethyl ether. These were all run simultaneously at a temperature of 58° C. The results are given in table IV. These results indicate that with this lot of petroleum ether there is no significant difference in the rate at which the two ethers are distilled from the oil.

The results of fourteen oil determinations from one large sample of leaves taken from a commercially sprayed grove are given in table V. This material was collected three months after application of the spray. The tests were made to determine the accuracy of the extraction method. These tests of twelve samples were each extracted for 20 minutes. They show a variation in the oil obtained ranging from 0.458 to 0.496 cc. per 100 gm. of dry tissue. The average for the twelve samples was 0.477 cc. The two samples extracted for 24 hours show a little more oil, 0.496 and 0.544 cc. respectively. This indicates that not quite all of the oil was removed from the leaves in 20 minutes.

RATE OF DISAPPEARANCE OF VARIOUS SPRAY OILS FROM LEAVES OF CITRUS

For this experiment five oils were used: Oronite Technical, Oronite X, Special 80, Oronite Cosmetic, and Oronite Mineral Seal, with specifications as given previously. Five large Valencia trees were sprayed with each oil, using the tank-mix method with a casein spreader and 2 per cent. oil.

TABLE V

OIL OBTAINED FROM SAMPLES OF VALENCIA ORANGE LEAVES SPRAYED COMMERCIALLY THREE MONTHS PREVIOUSLY

SAMPLE NO.	OIL IN 50 GM. LEAVES	OIL PER 100 GM. LEAVES	TIME EXTRACTED
	mm.	*cc.*	*min.*
1	10.1	0.486	20
2	10.1	0.486	20
3	9.8	0.470	20
4	10.3	0.496	20
5	9.5	0.458	20
6	9.5	0.458	20
7	10.1	0.486	20
8	10.0	0.482	20
			hrs.
9	10.3	0.496	24
10	11.3	0.544	24
			min.
11	9.7	0.468	20
12	9.9	0.476	20
13	10.0	0.482	20
14	10.0	0.482	20

Samples of leaves were collected from these trees at intervals and the oil content determined by the method already described. The results of these tests are given in table VI (graphically represented in fig. 11). This table gives the length of the column of oil in the neck of the test bottle in millimeters for each of the two tests of each sample. These tests show that practically all of the Mineral Seal oil had disappeared in 173 days while about 33 per cent. of the heavy Oronite Technical oil remained in the leaves.

These data (table VI, fig. 11) indicate that there is a rapid disappearance of all oils for the first three weeks. They show that the disappearance of oil after the first three weeks is relatively slow, and that there is still an appreciable quantity of the heavier oil in the leaves nearly six months after the application.

Collections from these same trees were made in which the material from the north side of the trees was kept separate from that on the south. The data are given graphically in figure 12.

It is evident from these data that the oil disappears more rapidly from the leaves on the south side of the tree than from those on the north side. There seems to be little if any difference in the rate of disappearance of the oil from the twigs on the two sides of the tree. These data also fail to show

TABLE VI

PETROLEUM OIL FOUND IN VALENCIA ORANGE LEAVES SPRAYED SEPTEMBER 8, 1930, WITH DIFFERENT OILS
LEAVES DRIED IN OVEN AT 80° C. WITH EXCEPTION OF LAST TWO COLLECTIONS WHICH WERE DRIED AT ROOM TEMPERATURE
25-GM. SAMPLES USED IN EACH TEST

DAYS AFTER SPRAY APPLICATION	ORONITE MINERAL SEAL		ORONITE COSMETIC		SPECIAL 80		ORONITE X		ORONITE TECHNICAL	
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2
	*mm.**	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	14.2	12.7	22.2	23.3	17.6	16.5	25.5	24.2	25.0	25.1
4	8.0	8 6	20.0	19.4	13.9	14.0	20.5	20.5	21.5	20.3
10	5 0	6 0	14.5	14.8	10.5	10.5				
15	4.3	4.4	11.7	12.8	9.1		15.0	15.3	17.0	17.5
22	3.7	3.5	11.6	11.5	7.2	7.2	12.8	11.6	15.7	14.5
39	3.6	3.2	11.0	11.7	5.8	6 1	10.8	11.5	15.2	
55	2.3		8.0	8.4	5.0	5.4	9.9	10.9	14.5	13.7
84	2.0	2.2	5.0	5.5	4.5	4.3	9.8	9.7	12.9	13.3
94	2.2	2.4	7.7	7.8	5.3	5.1	8.2	9.1	12.3	12.8
173	Trace		5.0		3 0		6.5	6.4	8.0	8.0

* mm. = length in mm. of oil column in Babcock milk test bottle.

any evidence that the disappearance of the oil from the leaves was due to a movement of the oil into the twigs.

In order to be more certain of the results in table I, another set of similar tests was run. Five navel orange trees were sprayed with each of the following sprays: 2 per cent. Oronite Technical Mixol; 2 per cent. Oronite

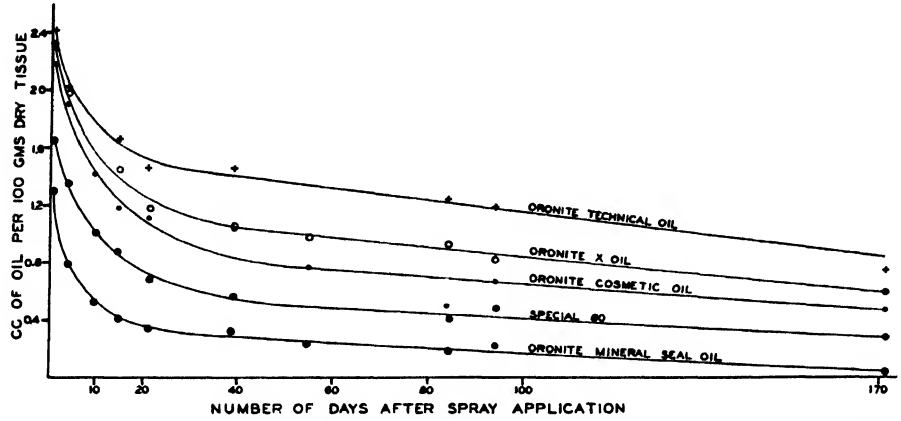


FIG. 11. Graph showing rate of disappearance of petroleum spray oil from Valencia orange leaves sprayed with 2 per cent. oil in tank-mix emulsion Sept. 9, 1930, to Feb. 28, 1931. Leaves dried at 80° C.

Technical tank-mix, using 4 ounces of blood albumin spreader per 100 gallons of spray; 2 per cent. Volck Concentrate; 2 per cent. Volck Medium; 2 per cent. Volck Light; 2 per cent. Oronite Mineral Seal tank-mix, using

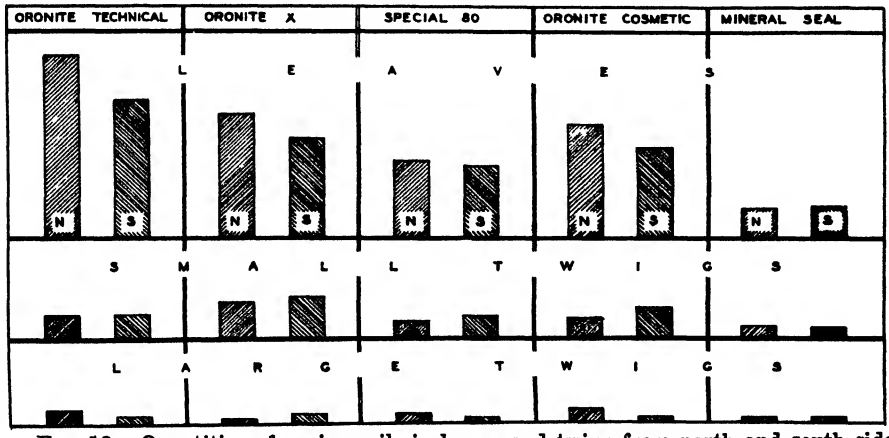


FIG. 12. Quantities of various oils in leaves and twigs from north and south sides of orange trees sprayed Sept. 8, 1930. Collections made Dec. 12, 1930.

4 ounces of blood albumin spreader per 100 gallons of spray. Five trees were left as checks. Samples of leaves were collected at intervals and dried in the laboratory at room temperature. The results of the tests are shown graphically in figure 13.

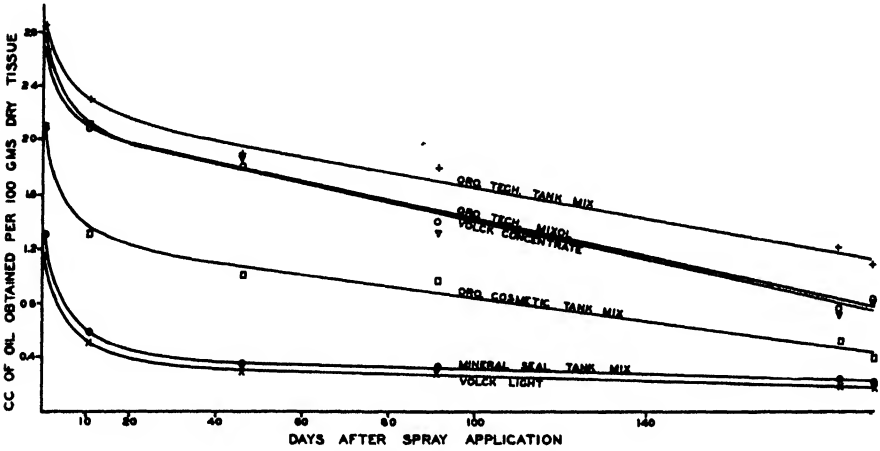


FIG. 13. Graph showing rate of disappearance of petroleum spray oil from navel orange leaves sprayed with 2 per cent. oil emulsions July 27, 1932, to Jan. 26, 1933. Leaves dried at room temperature.

The graphs in figure 13 show results remarkably similar to those in figure 11 except that the curves in figure 13 indicate that the oil did not disappear so rapidly during the first three weeks. This is no doubt due to the fact that the leaves used for the data given in figure 11 were dried in an oven at 80° C., while those used for figure 13 were dried at room temperature. It will be noticed also that the curves in figure 13 show that the rate of disappearance of Volck Concentrate and Oronite Technical Mixol is somewhat more rapid than that of the Oronite Technical tank-mix. After 192 days the Volck Concentrate and the Oronite Technical Mixol show 0.75 cc. of oil per 100 gm. of dried leaves, while the Oronite Technical tank-mix shows about 1.12 cc. of oil per 100 gm. of dried leaves (or about 50 per cent. more oil). It is believed that this slower disappearance of the tank-mix emulsion may be due to a difference in the size of the oil globules. The difference in size of oil globules may be caused by a higher surface tension in the tank-mix, which results in a smaller amount of oil surface exposed to the air in those tissues sprayed with tank-mix emulsions. Therefore tank-mix emulsions may have a slower evaporation rate.

Table VII gives the results of tests made on materials collected from commercial groves which had been sprayed with widely different materials

TABLE VII

OIL FOUND IN CITRUS LEAVES COLLECTED FROM COMMERCIAL GROVES

GROVE NO.	LOCATION	DATE SPRAYED (1931)	TIME AFTER APPLICATION	SPRAY MATERIAL	KIND OF TREES	DEPT. AGR. OIL GROUP NO.*	OIL PER 100 GM. LEAVES
			months				*cc.*
1	Whittier	Oct. 22	10	Volck Light Medium 1–2/3%	Valencia	2	0.28
2	Orange	Oct. 1	11	Standard 65 T.M.	"	2	Trace
3	Orange	Sept. 20	12	Orthol K. Lt. Med.	"	2	0.21
4	Whittier	Aug. 31	12	Volck Lt. Med. 2%	"	2	0.19
5	Riverside	July 12	12	Medona 65	Navel	2	0.19
6	"	July 22	12	Medona 65	"	2	0.22
7	"	July 26	12	Orange-ol	"	2	0.15
8	Orange	Oct. 31	10	Volck Med. T.M.	Valencia	3	0.33
9	Whittier	Sept. 17	11	Volck Med. 2%	"	3	0.25
10	Orange	Sept. 31	11	Volck Med. T.M.	"	3	0.32
11	Whittier	Aug. 31	12	Volck Med. 2%	"	3	0.33
12	Orange	Oct. 31	12	Volck Med. 2%	"	3	0.33
13	Whittier	Sept. 17	11	Volck Conc. 2%	Lemon	5	0.64
14	"	Sept. 18	11	Lemon Mixol 3%	"	5	0.62
15	"	Sept. 18	11	Del Monte Heavy 2%	"	5	0.57
16	"	Sept. 31	11	Volck Conc. 2%	"	5	0.68

* Classification of California State Department of Agriculture based on distillation:

51 to 63 per cent. of no. 2 oil distills over 636° F.

43 to 51 per cent. of no. 3 oil distills over 636° F.

0 to 29 per cent. of no. 5 oil distills over 636° F.

at different times and under different conditions. These data indicate that usually there is a small quantity of oil still on the trees at the end of one year in commercially sprayed groves, even when the lighter oils are used. There is a distinct difference in the quantities of oil left on the trees by the oils in the different groups, but within any one group the variation is less than might have been expected.

Figure 14 gives graphically some of the results of an experiment designed to determine whether there was any difference in the type of pene-

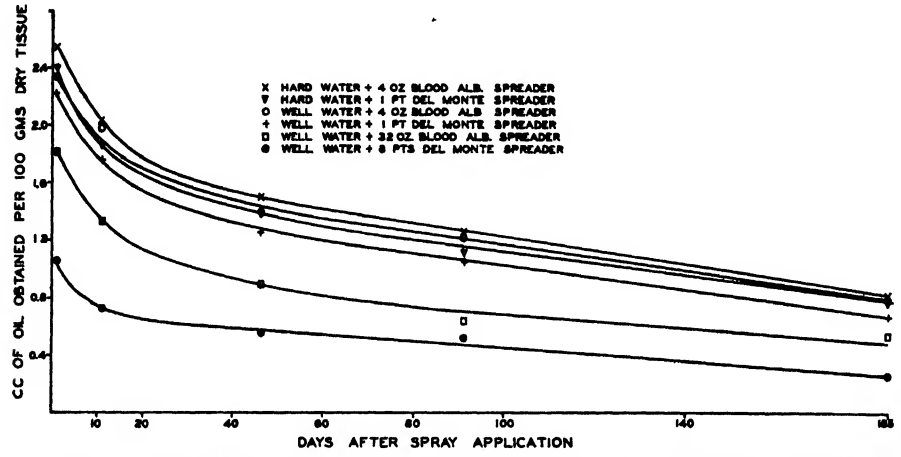


FIG. 14. Rate of disappearance of petroleum spray oil from Valencia orange leaves sprayed with 2 per cent. Oronite Technical oil July 27, 1932, to Jan. 26, 1933.

tration where widely differing quantities of spreader are used and where hard and moderately soft water are used in the emulsions.

Four large Valencia orange trees were sprayed with each of the emulsions used. Two per cent. of Oronite Technical oil was used in all of these sprays.

Two types of emulsifiers were used. The blood albumin spreader is recommended by the University of California (17) for use in the tank-mix emulsions. Four ounces of spreader per 100 gallons of water is the recommended amount. The other spreader used was the Del Monte spreader which is put out by the California Spray Chemical Corporation. This is a liquid spreader and it is recommended for use at the rate of 1 pint per 100 gallons of water. For the soft water the Riverside city water was used. This water has a conductivity of about $K \times 10^5 = 40$, or equal to about 0.0037 normal $CaCl_2$ (equivalent to 103 gm. $CaCl_2 \cdot 2H_2O$ per 100 gallons of water). This is rather soft water in comparison with most waters in southern California. For the hard water the same water with the addition of 537 gm.

of $CaCl_2 \cdot 2H_2O$ per 100 gallons was used. The addition of this salt brought the conductivity $K \times 10^5$ to about 225, which is about as hard as any water which would be used in southern California. These data indicate that the added "hardness" had some effect in causing a heavier deposit of oil by both emulsifiers, but the difference is not great.

The emulsions in which blood albumin was used deposited more oil than those in which the Del Monte spreader was used. This may have been due to the fact that the blood albumin used had been on hand for several months, and its solubility may have been less than that of fresh spreader. The emulsions in which eight times the normal quantity of spreader was used showed a marked reduction in the quantity of oil deposited. The one in which the Del Monte spreader was used deposited only about one-third as much oil as some of the other sprays. There is also a wide difference in the quantity of oil deposited by the 32 ounces of blood albumin and by the 8 pints of Del Monte spreader, the blood albumin depositing nearly twice as much oil.

Sections from leaves sprayed with the different emulsions showed no significant differences in the appearance of the oil in the tissues nor in its general location. There was considerable difference in quantity of oil-soaked area present, this difference being in accord with the results of the extractions. The oil-soaked areas in the leaves were limited to only very small places here and there on the edges of those leaves which were sprayed with emulsions containing an excessive quantity of spreader, while the others contained extensive oil-soaked areas sometimes covering nearly half of the leaf surface.

Comparison of results of histological studies with results obtained by oil extraction

Little can be determined by histological studies as to the quantity of oil in the tissues, since the distribution of the oil is so uneven throughout the different parts of the tree and even throughout the different parts of the same leaf or twig. In the more open parts of the plant, such as the spongy parenchyma of the leaf, the oil is more or less in large globules, while in the palisade tissues it is in very thin films between the closely fitting cells. It is difficult to compare under the microscope the quantities of oil in the two places.

The extraction of oils does not give much information as to the exact location of the oil, but it does give good indications as to the quantity present in any given tissue. Sometimes one may extract leaves which have been sprayed for as long as a year or more previously, and find measurable quantities of oil. It may be difficult to find oil in sections of these leaves

unless one allows the leaves to wilt, after which one may pick out those showing oil-soaked areas. Stained sections of these areas will show the presence of oil. Again, one may find a few leaves with small oil-soaked areas which when sectioned through the oil-soaked parts will show the presence of oil, but there may be such a small total area containing oil that in extracting only a trace of it will be found.

Summary and conclusions

1. A method is presented for the staining of sections of leaves, stems, and fruit of citrus trees by means of which the natural plant oils may be distinguished from the petroleum spray oils which have been applied to the trees.

2. The path of entrance and the location of the spray oils in the different tissues are discussed and illustrated.

3. A quantitative method for purifying and measuring the petroleum spray oil extracted from citrus tissues is described. This method has been used for the determination of the rate of disappearance of the petroleum oil from the trees.

4. The spray oils appear to enter the plant tissues by capillarity. When applied according to recognized spray practice the oil does not penetrate deeply into the plant tissue. It remains between the cells just under the epidermis of leaves, stems, and fruit. Only in the case of heavy applications does it penetrate to a depth of more than half a dozen plant cells beneath the epidermis. Under the usual conditions of application, no evidence has been found that the oil enters those plant cells which contain protoplasm. The less volatile portions of all except the lightest of the spray oils remain in the citrus leaves throughout their lifetime, often more than two years. In spite of the oil in these leaves they appear to remain healthy.

5. The petroleum oils are not found to be distributed evenly in the leaves, but are concentrated along the midrib and especially along the margin. Their distribution may be seen by the oil-soaked areas in leaves which have been allowed to wilt.

6. When sufficient quantities of oil are applied to citrus, the oil may penetrate deeply, even to the pith of twigs several centimeters in diameter.

7. No evidence has been found of any translocation or other movement of oils from leaves into twigs or from small twigs into larger twigs, except as the oils may migrate short distances between the cells by capillarity.

8. Extraction and measurement of the spray oil in citrus leaves indicate that with the heavier oils about one-third of the oil disappears during

the first three weeks after application, while with the lighter oils two-thirds to three-fourths of the oil may disappear during this time.

9. The data presented indicate that there is but little of a lighter spray oil left on a tree six months after its application, while as much an one-third of a heavier oil may be present after that length of time.

DEPARTMENT OF PLANT PHYSIOLOGY
UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BAILEY, I. W. The cambium and its derivative tissues. V. A reconnaissance of the vacuome in living cells. Zeitschr. wiss. Biol. Abt. B. Zeitschr. Zellf. und mikrosk. Anat. **10**: 651–682. 1930.
2. CALIFORNIA FRUIT GROWERS EXCHANGE. Handbook of citrus insect control for 1931. California Fruit Growers Exchange. Bull. 8. 1–24. 1931.
3. CONN, H. J. A method of detecting rennet production by bacteria. Jour. Bact. **7**: 447–448. 1922.
4. CONTOLEON, E., and JOANNIDES, G. S. Recherches expérimentales sur l'action de la paraffine liquide dans le tube digestif. Grèce. Méd. **28**: 2–4. 1926. (Biol. Abst. **1**: 11717.)
5. DAWSEY, LYNN H., and HAAS, A. J., JR. A method for determining the quantity of mineral oil retained by leaf surfaces after spraying. Jour. Agr. Res. **46**: 41–49. 1933.
6. DUFRENOY, J. Les modifications pathologiques de la structure des cellules végétales. Ann. de l'Inst. Nat. Agron. 2e Série Tome XXIII 39e volume de la collection. 1–104. 1930.
7. DUGGAR, B. M. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. **31**: 38–66. 1901.
8. ENGLISH, L. L. A method for determining the quantity of oil retained by citrus foliage after spraying. Jour. Agr. Res. **41**: 131–133. 1930.
9. GOLDSWORTHY, M. C., and SMITH, RALPH E. Studies on a rust of clingstone peaches in California. Phytopath. **21**: 133–168. 1931.
10. KELLY, VICTOR W. Effect of certain hydrocarbon oils on respiration of foliage and dormant twigs of the apple. Illinois Agr. Exp. Sta. Bull. 348. 371–406. 1930.
11. KNIGHT, H., CHAMBERLIN, J. C., and SAMUELS, C. D. On some limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol. **4**: 299–321. 1929.

12. Los Angeles County Agricultural Commission. Annual report for the year ending June 30, 1930. 46 pp. Los Angeles County, California.
13. McWhorter, F. P. Fungicidal value of oil sprays. Phytopath. **17**: 201–202. 1927.
14. Melhus, I. E., and Durrell, L. W. Studies on the crown rust of oats. Iowa Agr. Exp. Sta. Res. Bull. 49. 115–144. 1919.
15. Proescher, F. Oil red O pyridin, a rapid fat stain. Stain Technol. **2**: 60–61. 1927.
16. Smith, J. Lorrain, and Mair, W. Fats and lipoids in relation to methods of staining. Skand. Arch. Physiol. **25**: 247–255. 1911.
17. Smith, Ralph H. The tank-mixture method of using oil spray. California Agr. Exp. Sta. Bull. 527. 1932.

CARBOHYDRATE–NITROGEN RATIOS WITH RESPECT TO THE SEXUAL EXPRESSION OF HEMP

PAUL J. TALLEY

(WITH TWO FIGURES)

Introduction

Most of the recent work on the determination of sex in the higher plants has been done by cytologists and geneticists. These investigations have resulted in the chromosome theory of sex inheritance and sex determination, which is supported by much evidence in the dioecious forms of the higher plants.

Any adequate theory of sex inheritance and determination in the angiosperms must do more than account for the usual approximate 1:1 ratio of the sexes in dioecious plants. It must also explain the reversal of sexes within the so-called dioecious plants and the determination and development of both types of sporophylls on the same plant, as in the monoecious types. It should even explain the development of both types of sporophylls within one flower, as is the case with the majority of the angiosperms. The segregation of the microsporophylls and the macrosporophylls to individual plants should be considered the exception and not the general case. Any theory of sex determination based only on dioecious types is subject to many exceptions and criticisms.

The absence, or apparent absence, of allosomes in many dioecious plants and the frequency of reversal from the staminate to the pistillate condition, or *vice versa*, in forms in which allosomes have been reported, have led to differences of opinion as to the final importance of the "sex chromosomes" in sex determination in the dioecious angiosperms. The reversal of the sexes in dioecious plants indicates that sex determination and inheritance may result from the action of two different, but closely related, sets of factors in the development of these plants. The prevailing 1:1 ratio of the sexes indicates that a genetical tendency to produce one or the other type of flower is probably inherited in a strictly Mendelian manner. The evidence from the development of intersexes under certain environmental conditions shows clearly that the final display of the staminate or of the pistillate condition is determined by physiological as well as by genetic factors, even in dioecious plants. Probably the development of staminate and pistillate flowers is more dependent on physiological factors in monoecious than in dioecious plants. In plants with perfect flowers the development of stamens and pistils is probably chiefly dependent on physiological factors. Indeed it would be difficult to explain the determination of the

sex of the floral organs in monoecious plants and in plants with bisexual flowers on only a Mendelian basis, although genetic factors undoubtedly play an important part in determining the order of appearance and the arrangement of stamens and pistils in bisexual flowers and of pistillate and staminate flowers in monoecious species.

If the chromosome theory of sex inheritance is accepted for dioecious plants, it need not imply that sex determination is finally accomplished by means of the chromatin content alone. The observations in nature indicate that the potentialities for both types of spores are present at all times in all heterosporous angiosperm sporophytes, whether dioecious, monoecious, or perfect with respect to flower types. This has frequently been assumed. In that case dioecism would be considered as the suppression of one type of sporophyll on a given plant and monoecism would imply the suppression of one type of sporophyll only within a given region of the floral structures. The inherent nature to behave in this manner, under the usual conditions of growth, is probably controlled by genetic factors. Whether or not this is the case, there remains the need for an explanation of the controlling internal physiological mechanism that is functioning at the time of determination of staminate and pistillate structures. This internal physiological mechanism is at present the unknown factor. Regardless of our lack of knowledge concerning the nature of this physiological mechanism, it has been assumed to be the fluctuating factor which makes possible the reversal of the sexes in dioecious plants. This same concept can be applied to the development of only one type of fertile sporophylls in one region and of the other type of fertile sporophylls in another region of the same heterosporous plant. The physiological mechanism in question need not be fixed; perhaps it may be changed by an altered environment or by the metabolism of the plant.

Geneticists in general do not attempt to explain the mechanism by which a gene, or set of genes, brings about the manifestation of the particular morphological and physiological characters with which they are connected, but this manifestation is thought to be the result of a series of processes which begins with the interaction between the chromosome substance and the particular type and state of the cytoplasm within which the chromosomes are contained. Many genes are recognized which are influenced, or limited, in their actions by the conditions of the plant or of the tissue in which the character is expressed. If this is the case for genes influencing sexual expression, then it is obviously necessary to postulate a difference in the physiological conditions varying with time or location, or both, which makes possible the expression of the staminate or pistillate conditions in the ordinary monoecious plant or in a plant with perfect flowers. The genetic constitution is not known to be different in the stami-

nate and pistillate organs of these plants, with the possible exception of *Begonia* (**20**). Thus there is need for a study of the physiological differences associated with the development of stamens and pistils to explain the determination of sex in the higher plants. It is obviously difficult to obtain data which are conclusive in this respect. It will ultimately be necessary to determine whether or not the physiological differences represent the changes which accompany or follow the real determining physiological factors.

The problem of the physiological factors associated with the formation of staminate and pistillate structures respectively has not been directly attacked. Obviously the difficulties in making such a study on plants with bisporangiate flowers are very great. The problem should be less difficult in such monoecious plants as corn, and least difficult in a dioecious plant like hemp.

Review of literature

KLEBS (**11**) emphasized the complexity of the factors influencing the types of structures formed by plants. He considered the living cell as being influenced by three sets of factors: (1) the specific structure, (2) the internal conditions, and (3) the external conditions. His use of the term specific structure includes what might be termed the inherent composition of the cell. By varying the environmental factors in his studies on *Saprolegnia mixta* (**11**) and *Sempervivum funkii* (**10**), KLEBS showed clearly that the behavior of these organisms is not controlled by a fixed inner rhythm but is subject to control (within limits) by external factors.

Since the work of KLEBS, the most important advances in the mechanics of development and in our knowdge of the factors determining differentiation in the seed plants have fallen along two general lines. KRAUS and KRAYBILL (**12**) report a correlation between the carbohydrate and nitrogen content of the tomato plant and fruitfulness. The literature on carbohydrate-nitrogen ratios has become so extensive that no attempt will be made to review it here. The writer knows of no work pertaining to carbohydrate-nitrogen ratios in the two sexes of a dioecious angiosperm.

Another line of advance in our knowledge of the factors determining growth and reproduction in the angiosperms was initiated by GARNER and ALLARD (**6**) in their work on photoperiodicity. Probably the reaction of the plant to the proper photoperiod is only one means of producing an internal condition favorable to the formation of flowers and of influencing sexual reproduction. It should be possible to produce this same end result by altering one or more factors in the environment of the plant other than the photoperiod.

Some photoperiodic studies have been conducted on dioecious plants.

SCHAFFNER (**23, 25**), MCPHEE (**15**), and ADAMS (**1**) have varied the daily period of illumination on hemp. SCHAFFNER found that a decreased length of day is accompanied by an earlier flowering and an increase in the relative number of sex reversals. He reported that almost 100 per cent. of the staminate plants show some pistillate structures when flowering occurs in the middle of the winter. MCPHEE failed to obtain as high a percentage of reversals or intersexes as SCHAFFNER reported. ADAMS gave no data on this phase of the subject.

WESTER (**29**), in an account of the work of CIESIELSKY on sex determination in hemp, referred to the latter's conclusion that sex is normally determined by the age of the pollen at the time of pollination, but BESSEY (**2**) and BOSE (**3**) failed to find any support for this theory from their experiments on pollen age and sex.

The cytological aspect of sex inheritance in hemp has been studied by several workers. STRASBURGER (**28**) and MCPHEE (**14**) failed to find any apparent sex chromosomes, but SINOTÔ (**26**) and HIRATA (**9**) reported allosomes from studies on meiosis in staminate plants.

The genetical aspect of sex inheritance in hemp has been studied in breeding experiments by MCPHEE (**16**) and HIRATA (**8**). MCPHEE suggested that the genetical mechanism is of the XY type, the staminate plants being heterozygous for the factor or factors involved. The conclusions of MCPHEE are supported by the work of HIRATA.

It is generally agreed that the sexual expression of hemp can be modified in certain strains by environmental factors. MCPHEE (**16**), HIRATA (**7**), and BOSE (**3**) have discussed the frequency of sex reversals in different strains of hemp. CORRENS (**5**) has classified hemp as trioecious because the appearance of monoecious plants is so frequent in this species.

SCHAFFNER (**24**) has caused hemp plants to pass from a vegetative condition into a flowering condition and then back to a vegetative state for a prolonged period of time by changing the photoperiod. When flowering recurs the staminate plants frequently produce some intersexes. BOSE's (**3**) observations show that the reversals of sex are more common in the staminate plants. He found that the occurrence of intergrades in the field is limited almost completely to the flowers formed at the beginning or near the end of the period of flowering. These two observations support the view that conditions existing within the plant largely influence the types of flowers found at a given time. If the determining factors are physiological, one might expect that the conditions within the plant determining the formation of staminate flowers, or inhibiting the formation of pistillate flowers, on a genetically staminate plant would be gradually developed. One need not assume that these internal physiological factors remain fixed from the time of initiation of the first flower primordia until the cessation of flower formation.

PRITCHARD (**21**) obtained sex reversals in hemp by mutilating the plants, that is, by removing the floral branches or leaves. Covering the tips of the plants with manila bags was found to be equally effective in this respect. The injection of solutions of carbohydrates and nitrogenous compounds into the stem after mutilation seems to be of further help in causing the production of intersexes in some cases. BOSE (**3**) attempted to repeat this work, using the transpiration stream as a means of distributing the liquids through the plant instead of injecting them mechanically. Both investigators found that decapitation and covering the tips with bags are the best means of forcing sex reversals in the field. BOSE doubted the significance of his results since some intersexes appeared in his controls.

The metabolic differences between the sexes in dioecious angiosperms have not been investigated to any extent. SATINA and BLAKESLEE (**22**) applied several biochemical tests to certain Mucorales in an effort to distinguish metabolic differences between the heterothallic strains of this group of fungi. They applied similar tests to the leaves of some of the dioecious angiosperms, hemp being among those tested. They found that the Manoilov reaction, which is based on the reduction of potassium permanganate, is of a different order for the leaves of staminate and pistillate plants of hemp. They also reported a difference between the sexes in the natural color of the alcoholic extracts and in the oxygenase and the peroxidase activities, while the pH of the two types of plants is practically the same. They used only the leaves from staminate and pistillate plants. CAMP (**4**) reported that the catalase activity of reproductive organs, or structures closely associated with them, is higher in the case of staminate tissues than in the corresponding tissues of pistillate plants of the same species. The same relationship was reported by him for the catalase activity of the staminate structures or flowers in contrast to activity of the pistillate structures or flowers of monoecious plants or plants with bisexual flowers.

Many indications of physiological differences between staminate and pistillate angiosperms exist. Such differences are frequently assumed to be important in sex determination, but there is a noticeable paucity of data on the subject. The results here presented are the outcome of an attempt to ascertain some of the differences and similarities in staminate and pistillate plants of hemp.

Methods

Hemp seeds obtained from a commercial seed company were planted in soil trays on February 7 and transplanted into a deep soil bed in the greenhouse on February 22. The length of the photoperiod was increased to 15 hours by means of a battery of electric lights. A few male plants were beginning to flower on March 19. On April 2 typical male and

typical female plants were selected for analyses. The most mature flowers in the inflorescences of the staminate plants were beginning to shed pollen, but most of the flowers on each plant were immature at this time. A few of the flowers on the pistillate plants were fully developed, but no seed had been set. The average heights were 40 cm. for the pistillate plants and 52 cm. for the staminate plants. On April 5 another collection was made. These plants were in essentially the same stages of development as those collected three days earlier.

The plants were cut at the cotyledonary node at 4 P. M. and placed in separate weighed flasks for the determination of their fresh weights. Each plant was then cut into small pieces. The macerated tissue was mixed thoroughly and divided into two portions, each of which was weighed.

One of the portions was used for the determination of the percentage of dry matter in the sample and subsequently employed for the estimation of nitrogen. The other portion of each plant was used for the determination of carbohydrates. It was placed in 80 per cent. alcohol with a slight excess of calcium carbonate and refluxed for one hour to complete the extraction of the soluble carbohydrates. The procedure given by LOOMIS (**13**) was followed for the separation of the soluble and insoluble carbohydrates. All extracts were cleared by the use of natural lead acetate and deleaded by the use of potassium oxalate. Tests for reducing sugars were made on aliquots of the extracts obtained in this manner. Total sugars were determined on aliquots of these extracts after they had been hydrolyzed with 2 per cent. hydrochloric acid at 70° C. for 10 minutes and then neutralized with sodium carbonate.

That portion of the sample of each plant which was insoluble in 80 per cent. alcohol was hydrolyzed with 2 per cent. hydrochloric acid at the temperature of boiling water for two hours. The solution was then neutralized, cleared, and deleaded. Aliquots of this liquid were used for reducing sugar tests to estimate the acid-hydrolyzable polysaccharides present in each plant.

The carbohydrate fractions were determined by the use of Fehling's solution. The cuprous oxide was estimated by the volumetric iodide method given by MAHIN and CARR (**17**). Determinations were made on duplicate aliquots of each fraction of the carbohydrates. Each class of carbohydrates was expressed in terms of its glucose equivalent.

The nitrogen content of that portion of each plant used for dry weight determinations was estimated by the Kjeldahl method. Digestion was accomplished by the use of 20 cc. of concentrated sulphuric acid and 0.5 gm. of cupric sulphate. The distillate was received in normal acid and titrated with normal sodium hydroxide using methyl red as an indicator.

Results

The determinations of the dry weights show no differences between the percentages of moisture present in the aerial portions of the staminate and pistillate plants. There is a marked difference in the content of reducing sugars in the plants of the two sexes. The average content of reducing sugars expressed in terms of the fresh weight of the plants is 0.56 per cent. for the male plants and 0.23 per cent. for the female plants in the collection made on April 2. The same values for the collection made on April 5 are 0.81 for the males and 0.27 for the females. This striking difference still exists for the collections of both dates if the values are computed in terms of the dry matter. The differences in the contents of total sugars, expressed as glucose, also show the males to be higher than the females in the total quantity of the more soluble carbohydrates present on both dates. The results of these determinations are given in columns 2–6 inclusive of tables I and II.

The acid-hydrolyzable polysaccharides, expressed in terms of glucose, are more abundant on the average in the males than in the females, but the content of polysaccharides varies so much within the staminate plants that the average value for the group loses its possible significance (see columns 8 and 9 of tables I and II). The contents of the various classes of carbohydrates in the staminate and pistillate plants collected on April 5 are presented graphically in figure 1.

The average content of nitrogen is higher in the females than in the males, whether the values are computed on the basis of fresh weight or on the basis of dry matter. The results of the determinations of nitrogen are given in columns 12 and 13 of tables I and II.

The differences in the contents of the various carbohydrate fractions and of the nitrogen present in the staminate and pistillate plants are accentuated when the results are expressed in terms of the ratios of the carbohydrates to the nitrogen. The ratios of the reducing sugars to the nitrogen are higher for the staminate than for the pistillate plants. The same relationship holds for the ratios of the total sugars to the nitrogen. The average values of the ratios of the polysaccharides to the nitrogen are higher for the males than for the females; however, a few males have lower values than some few of the females for this ratio. The ratios of the various groups of carbohydrates and of the total carbohydrates to the nitrogen for the plants of both sexes are given in table III. The ratios for the plants collected on April 5 are presented graphically in figure 2.

Discussion

The determinations of the content of dry matter fail to show any differences between the sexes. If differences between the compositions of the

TABLE I

FRESH WEIGHTS AND PERCENTAGES OF DRY MATTER AND MOISTURE AND AMOUNTS OF REDUCING SUGARS, TOTAL SUGARS, POLYSACCHARIDES, TOTAL CARBOHYDRATES, AND NITROGEN AS PERCENTAGES OF FRESH AND DRY WEIGHTS OF STAMINATE AND PISTILLATE HEMP PLANTS HARVESTED APRIL 2

	Plant no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	13.1	21.03	78.97	0.79	3.78	1.38	6.58	3.01	14.3	4.39	20.9	0.81	3.88
2	11.3	20.81	79.19	0.48	2.34	1.43	6.90	3.01	14.5	4.44	21.4	0.80	3.84
3	15.8	20.86	79.14	0.43	2.07	1.24	5.94	2.78	13.3	4.02	19.2	0.90	4.29
4	5.7	20.59	79.41	0.47	2.25	2.13	10.27	2.81	13.6	4.94	23.9	0.85	4.12
5	3.4	24.49	75.51	0.40	1.62	3.47	14.05	5.70	23.1	9.17	37.2	0.77	3.11
6	17.6	21.12	78.88	0.78	3.68	1.45	6.88	2.75	13.0	4.20	19.9	0.67	3.16
Average	11.2	21.48	78.52	0.56	2.62	1.85	8.44	3.34	15.3	5.19	23.7	0.80	3.73
						Pistillate plants							
1	14.0	21.43	78.57	0.20	0.94	1.15	5.33	2.96	13.8	4.11	19.1	0.99	4.63
2	15.0	20.55	79.45	0.20	0.98	1.05	5.12	2.27	11.0	3.32	16.1	0.90	4.36
3	17.6	20.57	79.43	0.22	1.09	1.15	5.60	2.94	14.3	4.09	19.9	0.95	4.63
4	12.8	21.16	78.84	0.22	1.02	1.09	5.14	2.98	14.1	4.07	19.2	0.95	4.48
5	20.5	21.28	78.72	0.25	1.18	0.99	4.64	2.71	12.7	3.70	17.3	0.90	4.25
6	13.5	18.77	81.23	0.27	1.46	0.90	4.83	2.44	13.0	3.34	17.8	0.88	4.71
Average	15.5	20.63	79.37	0.23	1.11	1.06	5.11	2.71	13.2	3.77	18.3	0.93	4.51

TABLE II

FRESH WEIGHTS AND PERCENTAGES OF DRY MATTER AND MOISTURE AND AMOUNTS OF REDUCING SUGARS, TOTAL SUGARS, POLYSACCHARIDES, TOTAL CARBOHYDRATES, AND NITROGEN AS PERCENTAGES OF FRESH AND DRY WEIGHTS OF STAMINATE AND PISTILLATE HEMP PLANTS HARVESTED APRIL 5

	Plant no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	9.4	23.27	76.73	0.78	3.36	1.94	8.34	2.62	11.2	4.56	19.5	1.04	4.47
2	8.8	22.42	77.58	0.76	3.39	1.49	6.64	2.68	12.0	4.17	18.6	0.88	3.92
3	8.5	23.11	76.89	0.80	3.45	1.81	7.83	3.81	16.5	5.62	24.3	1.04	4.52
4	6.0	24.17	75.83	1.02	4.19	2.66	10.94	3.89	16.0	6.55	26.9	1.02	4.22
5	28.2	20.13	79.87	0.36	1.76	1.28	6.33	2.22	11.0	3.50	17.3	0.93	4.64
6	7.1	23.78	76.22	1.12	4.68	1.95	8.18	3.39	14.3	5.34	22.5	0.84	3.52
Average	11.4	22.81	77.19	0.81	3.47	1.86	8.04	3.10	13.5	4.96	21.5	0.96	4.22
						Pistillate plants							
1	16.7	23.98	76.02	0.26	1.08	1 17	4.88	2.63	11.0	3.80	15.9	1.12	4.68
2	21.7	17.18	82.82	0.20	1.16	0.99	5.78	1.88	10.9	2.87	16.7	0.92	5.33
3	9.2	25.31	74.69	0.31	1.21	1.21	4.77	3.09	12.2	4.30	17.0	1.22	4.82
4	18.5	20.52	79.48	0.26	1.28	0.97	4.74	2.10	10.3	3.07	15.0	1.00	4.87
5	19.0	20.97	79.03	0.34	1.63	1.09	5.19	2.37	11.3	3.46	16.5	0.98	4.70
6	25.5	23.11	76.89	0.23	1.01	1.13	4.90	2.68	11.5	3.80	16.4	1.12	4.82
Average	18.4	21.85	78.15	0.27	1.23	1.09	5.04	2.46	11.2	3.55	16.3	1.06	4.87

sexes exist, they are to be found in the compositions of the dry matter and not in the percentages of dry matter.

The analyses show several striking differences in the compositions of the dry matter of the sexes. The average content of carbohydrates is higher in the males than in the females. The significance of the average values is decreased by the fact that the lowest values for individual staminate plants

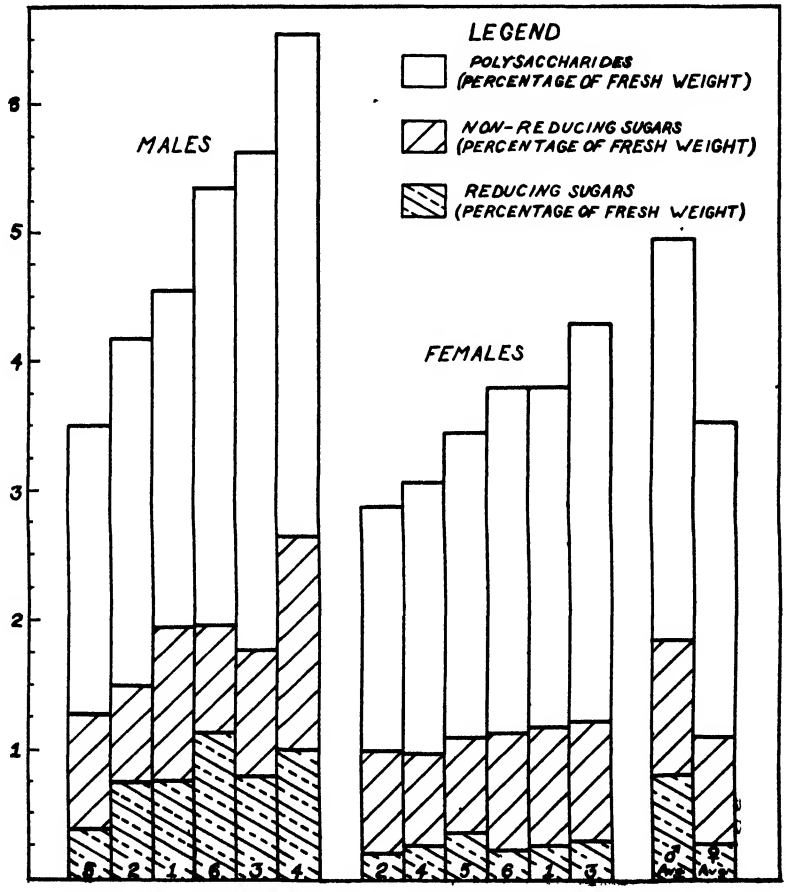


FIG. 1. Polygons showing carbohydrate contents of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

are less than the highest values for a few of the pistillate plants. Tables I and II show that in this case one is not only comparing staminate and pistillate plants but is also contrasting the largest staminate plants and the smallest pistillate plants. Usually the pistillate plants have a much greater total fresh weight than the staminate plants at the time of flowering. There are

TABLE III

RATIOS OF CARBOHYDRATE FRACTIONS TO THE NITROGEN

PLANT NO.	REDUCING SUGARS/ NITROGEN	TOTAL SUGARS/ NITROGEN	POLYSACCHARIDES/ NITROGEN	TOTAL CARBOHYDRATES/ NITROGEN
		Staminate (April 2)		
1	0.98	1.70	3.70	5.40
2	0.60	1.79	3.77	5.56
3	0.48	1.38	3.10	4.48
4	0.55	2.50	3.31	5.81
5	0.52	4.51	7.42	11.93
6	1.16	2.17	4.11	6.28
Average	0.71	2.34	4.24	6.58
		Pistillate (April 2)		
1	0.20	1.16	2.99	4.15
2	0.22	1.17	2.52	3.69
3	0.23	1.21	3.09	4.30
4	0.23	1.15	3.14	4.29
5	0.28	1.09	2.99	4.08
6	0.31	1.02	2.76	3.78
Average	0.25	1.14	2.91	4.05
		Staminate (April 5)		
1	0.75	1.86	2.51	4.37
2	0.86	1.69	3.05	4.74
3	0.77	1.74	3.66	5.40
4	1.00	2.60	3.80	6.40
5	0.38	1.37	2.38	3.75
6	1.33	2.32	4.05	6.37
Average	0.85	1.93	3.24	5.17
		Pistillate (April 5)		
1	0.23	1.04	2.35	3.39
2	0.22	1.09	2.05	3.14
3	0.25	0.99	2.53	3.52
4	0.26	0.97	2.10	3.07
5	0.35	1.11	2.41	3.52
6	0.21	1.02	2.39	3.41
Average	0.25	1.04	2.31	3.34

individual exceptions to this rule which probably can be accounted for in part by the lack of genetical uniformity in the seeds.

In contrast to the percentages of total carbohydrates, the percentages of total sugars not only show a higher average value for the staminate

plants in contrast to the average value for the pistillate plants, but there is no overlapping in the quantities of carbohydrates soluble in 80 per cent. alcohol present in the individual plants of the two sexes. The lowest value for any of the males is 1.24 per cent. of the fresh weight, while 1.21 is the highest percentage for any one female plant. This difference in the quantities of total sugars in the plants of the opposite sexes is found to be due almost entirely to the quantities of reducing sugars. This class of carbohydrates

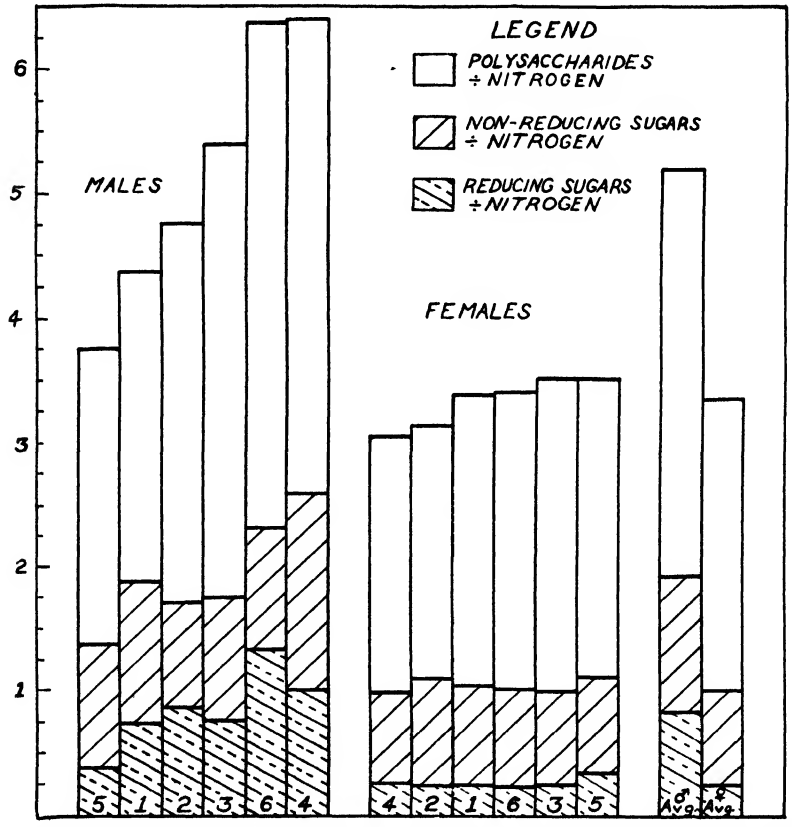


FIG. 2. Polygons showing carbohydrate-nitrogen ratios of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

presents the most contrasting difference between the chemical compositions of the sexes of hemp under the conditions in which these plants were grown. The average values for the reducing sugars, expressed as percentages of the fresh weight, are from two to three times as high for the males as for the females. The ranges of variation within the two sexes cover two separate

regions. Although there is a difference in the quantities of total sugars in the two sexes, it is based chiefly on the differences in the reducing sugars and not on the quantities of non-reducing sugars. Figure 1 presents these facts graphically and also indicates the ranges of variability in the contents of carbohydrates of the two sexes.

The pistillate plants are relatively constant; that is, the analyses show little variation in the percentages of reducing and total sugars in these plants. The situation existing within the staminate plants is different. The quantities of reducing and total sugars fluctuate from values slightly greater than those for the highest pistillate plants up to values clearly of a different order.

The higher content of carbohydrates and the wider range of fluctuation in all classes of carbohydrates in the staminate plants, in contrast to the condition in the pistillate plants, are very suggestive. One is inclined to consider that the presence of the greater quantities of reducing and total sugars in the males is not a result of the inability to synthesize polysaccharides, since they too are usually more abundant in the staminate plants. Nor is one justified by these analyses alone in assuming that the polysaccharides have been digested more rapidly in the males than in the females. Regardless of the causes of the differences in the quantities of the various fractions of carbohydrates in the sexes, the constancy of the females is in keeping with the later behavior of the plants. The females flower more gradually and apparently retain their vegetative vigor for a longer period of time than do the males. The staminate plants usually turn yellow and die rapidly after flowering. The greater variability in the content of carbohydrates in the males and their early senescence stand in contrast to the small amount of variability in the carbohydrates and the slow loss of vegetative vigor in the females, when both sexes are growing under early spring conditions in the greenhouse.

The results of the nitrogen analyses are not so significant. The pistillate plants have a larger average percentage of their fresh weights composed of nitrogen than have the staminate plants. However, the values for the individual staminate and pistillate plants do not fall into two groups as in the case of the reducing sugars. It is not advisable to attempt any detailed evaluation of the nitrogen determinations, since they include all of the reduced forms of nitrogen within the plants. It is probable that most of this nitrogen is present in the plant in complex organic molecules.

The ratios of the various classes of carbohydrates to the nitrogen present some interesting differences between the sexes (table III and fig. 2). The ratios of the reducing sugars to the nitrogen again show the females to be less variable than the males. The ratios are much greater for the staminate plants than for the pistillate plants; the average values for the males

are about three times as great as are those for the females. The greater ratios of the males are due chiefly to their higher content of reducing sugars but are partly the result of their lower content of nitrogen. This same situation exists in the ratios of the total sugars to the nitrogen, but the differences between these ratios for the sexes are not so great. It is evident that the main differences in the ratios of the total sugars to the nitrogen are chiefly the result of the differences in the ratios of the reducing sugars to the nitrogen. The constancy of the females is again evident in these ratios, while the males continue to show a greater variability.

The ratios of the polysaccharides to the nitrogen show that the males have a higher average ratio than the females, but the ranges of variability in these two groups overlap. These ratios obviously are not so significant as the ratios of the sugars to the nitrogen in differentiating between the sexes of hemp grown under the conditions of these experiments. The combinations of these ratios for the different plants, that is, the ratios of the total carbohydrates to the nitrogen, give higher average values for the staminate plants than for the pistillate plants. The lowest ratio for a staminate plant collected on April 2 is higher than the highest value for any pistillate plant collected on that date. The same relationship exists for the ratios of the staminate and pistillate plants collected on April 5. The ratios are greater for the plants collected on April 2 than they are for the collection of April 5. This is partly the result of a lower nitrogen content in the plants of both sexes collected on April 2 and a lower content of polysaccharides in the plants collected on April 5.

From the preceding discussion it is evident that the contents of carbohydrates and nitrogen are different in staminate and pistillate hemp plants at the time of flowering, when they are grown under the conditions of these experiments. These differences are seen to be the result of a relatively high content of sugars and a relatively low content of nitrogen in the staminate plants. These facts can be interpreted to explain the early senescence of the staminate plants and the retained vegetative vigor of the pistillate plants of greenhouse hemp. KRAUS and KRAYBILL (**12**) and others have shown that the relationships between the carbohydrates and nitrogenous materials are largely the determining factors in changing from the vegetative to the fruiting state in several bisporangiate angiosperms. The results obtained in these analyses of hemp plants in the early stages of flowering do not necessarily show any relationship to the quantities of these substances which were present before the plants began flowering, but it is evident that these staminate and pistillate plants have different carbohydrate-nitrogen ratios at the time of flowering. It remains to be determined whether the differences between the sexes in these ratios are causal physiological factors connected with the formation of stamens and pistils, or

whether they are merely accompanying factors. It does not necessarily follow that the conditions in the meristematic regions of the staminate and pistillate plants at the time of flower formation are properly reflected by the analyses of the whole aerial portions of the plants.

MURNEEK (**18**) has shown that tomato plants grown with a shortage of nitrogen turn yellow and decline vegetatively. This condition can soon be overcome by furnishing the plant available nitrogenous compounds. The staminate hemp plants, soon after they flower, act similarly to the nitrogen-starved tomato plants. A difference exists chiefly in the fact that the staminate hemp plants continue to decline even though they have the same source of nitrogen compounds as have the pistillate plants. They are apparently exhausted by the flowering process to the extent that they are unable to continue the assimilation of nitrogen rapidly enough to regain their lost vegetative vigor, as does the tomato plant.

The spider flower (*Cleome spinosa*) yields some interesting information with respect to types of flowers formed and the quantity of nutritive materials present. STOUT (**27**) has described the development of alternating whorls of fertile and sterile flowers in this bisporangiate angiosperm. MURNEEK (**19**) has altered the nitrogen available to this plant and finds intermittent sterility in plants with an abundance of or with a shortage of available nitrogen in the soil. MURNEEK found a difference in the growth of the plants and in the frequency of the alternating whorls of sterile and fertile flowers, but he was unable to cause the plant to overcome the tendency to form alternately fertile and sterile flowers by changing the quantity of nitrogen available to its roots. The removal of flowers containing fertile pistils has a direct effect on the type of flowers produced in the meristematic region of the spike. The percentage of the flowers able to set seed was increased greatly by this procedure. The intermittent sterility in the spider flower results from the alternation of whorls of flowers with aborted stamens and large functional pistils with whorls of flowers containing aborted pistils and large functional stamens. Thus the functional sex of the flowers of *Cleome spinosa* varies with some internal regulating condition determining the development of the essential floral parts. Since the removal of fertile flowers and fruits increases the width of the zones of fertile flowers being formed nearer the apex of the inflorescence, it seems that the formation of functionally pistillate flowers and the maturation of fruits both require similar, or the same, substances from the plant. Either these substances are not present in sufficient quantities or else they are not conducted to all parts of the inflorescence rapidly enough to permit a continuous formation of fertile flowers and the simultaneous maturation of fruits.

Since the removal of fruits is known to allow the nitrogenous compounds

to accumulate in certain plants (**18**), it is probable that the fertility of the flowers produced by *Cleome spinosa* is partly dependent upon the nitrogen supply available in the tissues in close proximity to the flower primordia. If this is true for the spider flower, one might consider that the functionally staminate flowers of *Cleome spinosa* were formed from meristematic regions lower in certain nitrogenous elements, or with a higher carbohydrate-nitrogen ratio, than the regions forming functionally pistillate flowers. The condition in hemp plants might be essentially the same except that the usual presence of aborted stamens or pistils within the flowers is not encountered. Since these analyses include the whole aerial portions, it is not safe to assume positively that the formation of stamens takes place in tissue with a higher carbohydrate-nitrogen ratio, while pistils are formed in a corresponding tissue with a lower carbohydrate-nitrogen ratio. If this were definitely proved, it would not necessarily follow that the sexual nature of the flower depended entirely on the quantities of these metabolic materials; but it is interesting to note this possible relationship between the sexual nature of the plant and the quantities of sugars and nitrogenous compounds present.

A further investigation into other aspects of the physiological differences and similarities between the sexes in hemp and in other plants is needed before one can properly evaluate the true significance of the carbohydrate-nitrogen ratios with respect to sex determination.

Summary

1. The aerial portions of staminate and pistillate plants of hemp were analyzed at the time they were coming into flower. Determinations were made of the total fresh weight of the individual plants and of the moisture, reducing sugars, total sugars, polysaccharides, and reduced forms of nitrogen.

2. Little if any difference in the percentages of moisture and dry matter exists between the sexes of hemp.

3. Staminate plants have higher average percentages of total carbohydrates, polysaccharides, and sugars than pistillate plants under the conditions of these experiments.

4. The reducing sugars are much more abundant in the staminate plants than in the pistillate plants.

5. Nitrogen is relatively more abundant in the pistillate plants than in the staminate plants.

6. The carbohydrate-nitrogen ratios of the sexes are discussed with respect to the differences in the sexual expressions and the habits of growth of the plants.

Acknowledgment is made of the encouragement and suggestions given by Professor W. F. LOEHWING of the University of Iowa during the early part of this investigation. Appreciation is expressed to Professor J. B. OVERTON of the University of Wisconsin, for his kind criticisms and advice in the preparation of this paper.

DEPARTMENT OF BIOLOGY
TEXAS AGRICULTURAL AND MECHANICAL COLLEGE
COLLEGE STATION, TEXAS

LITERATURE CITED

1. ADAMS, J. Duration of light and growth. Ann. Bot. **38**: 509–523. 1924.
2. BESSEY, E. A. Effect of the age of pollen upon the sex of hemp. Amer. Jour. Bot. **15**: 405–411. 1928.
3. BOSE, R. D. A study of sex in the Indian hemp. Agr. Jour. India **25**: 495–507. 1930.
4. CAMP, W. H. Catalase activity and sex in plants. Amer. Jour. Bot. **16**: 221–224. 1929.
5. CORRENS, C. Bestimmung, Vererbung und Verteilung des Geschlechtes bei den höhern Pflanzen. Handb. Vererbungswiss. **2**: 1–138. 1928.
6. GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.
7. HIRATA, K. Sex reversal in hemp. Jour. Soc. Agr. For. Sapporo **16**: 145–168. 1924.
8. ————. Sex determination in hemp. (*Cannabis sativa* L.). Jour. Genetics **19**: 65–79. 1927.
9. ————. Cytological basis of the sex determination in *Cannabis sativa* L. Japanese Jour. Genetics **4**: 198–201. 1929.
10. KLEBS, G. Ueber die Nachkommen künstlich veränderter Blüten von Sempervivum. Sitzber. Heidelb. Akad. Wiss. (Math.-Nat. Kl.) **5**: 1–32. 1909.
11. ————. Alterations in the development and forms of plants as a result of environment. Proc. Roy. Soc. London **B82**: 547–558. 1910.
12. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
13. LOOMIS, W. E. *et al.* The determination of soluble carbohydrates. Plant Physiol. **2**: 195–204. 1927.

14. McPHEE, H. C. Meiotic cytokinesis of *Cannabis*. Bot. Gaz. **78**: 335–341. 1924.
15. ————————. The influence of environment on sex in hemp, *Cannabis sativa* L. Jour. Agr. Res. **28**: 1067–1080. 1924.
16. ————————. The genetics of sex in hemp. Jour. Agr. Res. **31**: 935–943. 1925.
17. MAHIN, E. C., and CARR, R. H. Quantitative agricultural analysis. McGraw-Hill Book Co., New York. 1923.
18. MURNEEK, A. E. Effect of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon esculentum* Mill.). Plant Physiol. **1**: 3–56. 1926.
19. ————————. Physiology of reproduction in horticultural plants. II. The physiological basis of intermittent sterility with special reference to the spider flower. Missouri Agr. Exp. Sta. Res. Bull. 106. 1927.
20. PASTRANA, MARIA D. Sporogenesis and sex determination in *Begonia schmidtiana*. Amer. Jour. Bot. **19**: 365–384. 1932.
21. PRITCHARD, F. J. Change of sex in hemp. Jour. Hered. **7**: 325–329. 1916.
22. SATINA, SOPHIA, and BLAKESLEE, A. F. Differences between sexes in green plants. Proc. Nat. Acad. Sci. **12**: 197–202. 1926.
23. SCHAFFNER, J. H. The influence of relative length of daylight on the reversal of sex in hemp. Ecol. **4**: 323–334. 1923.
24. ————————. Further experiments in repeated rejuvenations in hemp and their bearing on the general problem of sex. Amer. Jour. Bot. **15**: 77–85. 1928.
25. ————————. The fluctuation curve of sex reversal in staminate hemp plants induced by photoperiodicity. Amer. Jour. Bot. **18**: 424–430. 1931.
26. SINOTÔ, Y. On the chromosome number and the unequal pair of chromosomes in some dioecious plants. Proc. Imp. Acad. Tokyo **4**: 175–177. 1928.
27. STOUT, A. B. Alternation of sexes and intermittent production of fruit in the spider flower (*Cleome spinosa*). Amer. Jour. Bot. **10**: 57–66. 1923.
28. STRASBURGER, E. Über geschlechtbestimmende Ursachen. Jahrb. wiss. Bot. **48**: 427–520. 1910.
29. WESTER, P. J. The determination of sex. Jour. Heredity **5**: 207–208. 1914.

LOSSES TO THE CORN CROP CAUSED BY LEAF INJURY

GEORGE H. DUNGAN

(WITH SIX FIGURES)

Introduction

The corn plant as it is grown in the corn belt is subject (1) to accidental injuries by man and his implements; (2) to destruction of tissues by insects and rodents; and (3) to rough treatment by nature in the tearing and twisting by strong winds, and in the beating and bruising action of rain and hail. This report deals with losses due to mutilations of the type caused by hailstorms.

Unfortunately hail does not injure certain rows of corn and leave the adjoining rows uninjured so that losses can be accurately compared and measured. Definite information of this kind can be obtained only from planned experiments. It is difficult if not impossible, however, to treat corn plants artificially in a way that accurately duplicates the injury caused by natural hailstorms. Notwithstanding the element of inaccuracy, experiments of the type here described are believed to be the best means of extending our knowledge of the degree of functional derangement due to injuries of a definite type and severity.

Nature of injury caused by hail

Hailstorms damage small corn plants mainly by shredding and beating off the blades. Large hailstones, if they strike small plants near the surface of the soil, may break them at the ground level.

With large plants hail causes injury by splitting the blades and by tearing away entire blades or portions of blades. Hailstones also bruise the stalks with a consequent interference with the movement of materials within the plant (fig. 1). If the hail occurs at pollination time, it may seriously interfere with normal fertilization of the kernels through the destruction of the functioning organs of the tassels and silks. Storms later in the season may result in direct damage to the developing ears. These may be knocked from the plant by large hailstones striking the supporting shank, or more frequently the corn on the cob may be bruised through the husk. Sometimes the impact is so great as to mash the immature kernels. Molds usually follow such injuries, especially if the ear is in the blister or milk stage.

Probably the greatest loss occasioned by hailstorms comes from injury to the leaves. Losses from blade injury are more difficult to estimate than

losses due to ear bruising. For this reason the experiments reported here were confined almost entirely to the leaf-injury and blade-removal phases of the problem.

According to a summary of the reports made by crop correspondents of the U. S. Department of Agriculture, the annual loss to the corn crop

FIG. 1. Corn plants injured by severe natural hailstorm on July 22, 1931. Most of the emerging tassels were either destroyed or so badly injured that they could not function in pollen production. Blades and sheathes have been cut away from plant on right to show the hailstone bruises on the stalk.

caused by hail in the United States averaged 0.6 per cent. during the period 1909–1924 (14). Even though this is a small percentage loss, it is considerable when expressed in bushels. This average loss of 0.6 per cent. is the result of a relatively few farmers suffering severe losses, sometimes approaching 100 per cent.

VALGREN (15) shows that the geographic division of the country including Illinois, Indiana, Michigan, Ohio, and Wisconsin suffered, as a 10-year average for 1909–1918, the smallest reduction in corn yield due to hail of any similar sized section of the United States. Although this reduction amounts to only 0.25 per cent. of the total crop, or 2,800,000 bushels, nevertheless, taken from a few growers, it represents a serious loss.

Experiments of other investigators

In some localities it is, or used to be, a common practice to cut off the corn plant just above the ear and use the cured tops for live-stock feeding. Topping is occasionally accompanied with stripping of the blades from that portion of the stalk below the ear. Stripping is sometimes practiced without topping. In order to obtain the tops and blades when they are high in feeding value, they are usually taken from the plant when the grain reaches the glaze or early dent stage.

CONNELL (1), TRACY and LLOYD (13), NEWMAN (9), and HUNT (8) found that the removal of green blades by such practices as topping and stripping was associated with a reduced yield of grain and frequently this loss in yield of corn was not compensated for by the value of the forage secured.

DIBBLE and MARSTON (3) topped corn to determine the value of this method as a means of controlling the European corn borer. As an average of tests covering two seasons, removing the tops just above the ear was followed by a 39 per cent. reduction in yield of grain. Topping 10 to 12 inches above the ear resulted in a 12 per cent. drop in yield, and topping 20 to 24 inches above the ear lowered the yield 5 per cent.

SAYRE, MORRIS, and RICHEY (11) found that the reduction of the leaf area of corn plants was associated with a decrease in the total sugar content of the stalk.

STEGGERDA (12) found that corn plants which had been injured produced less grain than uninjured plants, and the reduction in yield increased with the increasing number of blades removed.

CULPEPPER and MAGOON (2), DUNGAN (4, 5), ELDREDGE (6), and HUME and FRANZKE (7) found that the removal of all blades from corn plants at tasseling time produced the greatest reduction in yield. The greatest injury to the quality of grain resulted from defoliations made just after the silks had turned brown. Splitting and shredding the blades and breaking the midribs, or otherwise mutilating the blades if they were not entirely severed from the plant, did not result in such great yield reductions as did leaf pruning. It seemed that no matter into how many shreds the blades were torn, as long as the parts were attached to the plant they were capable of functioning in grain formation.

Methods

In the first artificial hailing experiments at Illinois, the hail-like injury was inflicted with a whip made of a bundle of 20-inch lengths of baling wire By striking the blades and stalks with the end of this whip, a wound resembling both a bruise and a tear was produced

Since it was believed that the main source of injury from hail was probably the reduced blade area, most of the later treatments involved leaf

FIG 2 Plants from which three, eight, and all blades were removed with hand shears

removal, either by jerking the blade from the sheath by hand, or by clipping them off with hand shears (fig 2).

When it was desired to remove one average blade from each plant in a certain row, the lowest green blade was clipped from the first plant, the

second blade from the second plant, the third blade from the third plant, and so on for twelve plants, twelve being the average number of blades on the plants. The process was repeated beginning with the thirteenth plant, from which the lowest blade was removed.

Two blades were removed from each plant by cutting off the first and sixth from the first plant, the second and seventh from the second, the third and eighth from the third, and so on. Another series was treated by removing three blades from each plant, another by removing four, and so on to the last row, which had all blades removed. These treatments were administered at different stages in the plant's development. The appearance of the plants with three, eight, and all blades removed is shown in figure 2.

Other types of mutilation were (a) clipping off the tip half of the blades; (b) removal of one side of the blade by splitting it longitudinally by the side of the midrib; (c) stripping the sides of the blades from the midrib but leaving them attached to the plant at their bases; (d) cutting each side of the blade to the midrib in 4-inch segments and removing alternate 4-inch pieces; (e) breaking the midrib of all blades about 3 inches from the stalk; and (f) cutting the blades crosswise to the midrib on both sides about 3 inches from the plant (fig. 3).

The corn used in the tests conducted during the period of experimentation, 1925–1928, was a uniform strain of open-pollinated Reid Yellow Dent. Alternate rows were treated, leaving untreated rows as checks. In 1929 a hybrid corn known as F_1365, developed by J. R. Holbert of the U. S. Department of Agriculture, was used. Only hills containing two plants of apparently equal size and stage of development were chosen. The plant occupying the north or west position in the hill was treated by removing the desired number of blades with hand shears. The other plant was left untreated to serve as a check. At harvest time each ear was husked, individually tagged, and stored in a room at 100°–110° F. for curing. When the ears reached a uniform moisture content, they were weighed, measured, and shelled. Also the grain was weighed and the test weight per bushel determined.

In a part of these experiments, an attempt was made to prevent excessive loss of water from the plants through wounds by administering the treatments during cloudy weather or late in the afternoon. This precaution was not taken in all cases, and it is probably of little consequence, for cutting down the transpiration surface occasioned by blade removal would likely lower the rate of water loss from plants much more than the wounds would increase it. WYLIE (17) has reported that injured leaves develop protective barriers promptly, thus checking evaporation and preventing fungal invasion.

Experimental results

Influence of stage of development on injury

Corn seedlings may be treated roughly with little or no serious injury On June 15, 1929, a rain of 0 32 inch was followed by a hailstorm of short duration. Hailstones the size of marbles fell with such force that they dented the soil in the corn field 0 5 inch in depth Some of them struck the corn plants, tearing the blades severely. Others hit the plants low on the stalk and snapped them off The plants at that time were about

Fig 3 Types of blade injury used in experiments to determine influence of leaf mutilation upon grain yield *A*, outer half of each blade removed; *B*, one side of each blade removed, *C*, sides of blades stripped from midrib, *D*, alternate 4 inch segments of blades on each side of midrib removed, *E*, midribs broken about 3 inches from their bases, *F*, blades cut to midrib on both sides about 3 inches from their bases

5 inches tall Within two days following the injury, new shoots were plainly visible These plants grew rapidly, although they were somewhat undersized throughout the season and ripened late

In order to secure data on recovery following such injury, plants were cut off level with the soil on June 22 and July 2. The average height of the plants at the time of treatment, the number that recovered, and the percentage reduction in yield of grain are presented in table I.

From the results it appears that young corn plants rapidly grow beyond the stage in which they have ability to produce new shoots when the tops are removed. The one plant that survived the treatment on July 2 was

TABLE I

EFFECT OF CUTTING OFF SMALL CORN PLANTS FLUSH WITH SURFACE OF SOIL AT TWO DIFFERENT STAGES OF DEVELOPMENT

DATE OF TREATMENT	AVERAGE HEIGHT TO TIP OF LONGEST BLADE	NUMBER OF PLANTS IN TEST	NUMBER OF PLANTS THAT RECOVERED	NUMBER OF PLANTS THAT PRODUCED GRAIN	REDUCTION IN YIELD OF SHELLED GRAIN COMPARED WITH UNTREATED CHECK
	m.				%
6/22	12.0	29	22	15	87.7
7/2	25.6	23	1	0	100.0

the smallest plant of the twenty-three in the test. It was only 19 inches tall when cut off. All the plants that were 20 inches tall at the time of treatment failed to produce new shoots.

WEIGERT (**16**) observed that young corn plants were much more sensitive to injury from hail than were older ones, for they are more readily

TABLE II

REDUCTION IN YIELD OF GRAIN ASSOCIATED WITH REMOVAL OF ALL BLADES FROM CORN PLANTS AT DIFFERENT STAGES OF DEVELOPMENT

STAGE	REDUCTION OF GRAIN YIELD IN			
	1926	1928	1929	AVERAGE
	%	%	%	%
Vegetative (plants 4 feet tall)			89.34	89.34
Tassel emerging			99.69	99.69
Ear shoot emerging			100.00	100.00
Fresh silk	95.50		98.61	97.06
Early blister		85.76		85.76
Blister	72.94	77.00	76.55	75.50
Early milk	54.55	59.40		56.98
Milk			50.18	50.18
Late milk	26.49			26.49
Glaze	16.95			16.95
Early dent		+1.15	10.14	4.50
Dent		+1.15	4.94	1.90

broken off. In later stages of development the injury consists mainly in the slitting of leaves, and only the extraordinarily severe strokes of hail break the stalks.

In order to test the ability of corn plants to recover from severe hail injury, all blades were removed at twelve different stages of growth, beginning when the plants were 4 feet tall and ending when the grain was well dented. The results, covering a 3-year period, expressed in terms of percentage reduction in yield of shelled corn, are presented in table II.

The greatest yield reduction occurred when all blades were removed during the period between tassel emergence and the fresh silk stage. Treatment earlier than this was not so detrimental to yield because some new leaves are unrolled with the elongation of the tassel-bearing stem. The data obtained suggest that the curve showing the percentage reduction in yield due to removal of all blades at successive stages of growth would start at zero for treatment soon after plumage emergence, and would mount gradually, reaching approximately 90 per cent. at the late vegetative stage, as indicated by the dotted line in figure 4. According to data secured, the

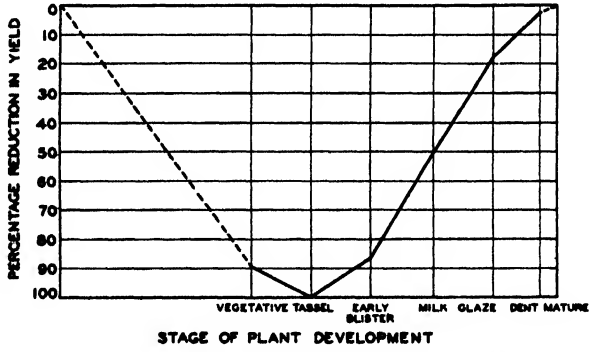


FIG. 4. Reduction in yield of corn following removal of all blades at different stages of plant and grain development (solid line drawn from data; broken line represents an estimation).

loss of grain reached a maximum of 100 per cent. for treatment at pollination time and dropped gradually as the grain developed, reaching the zero point at complete maturity (fig. 4).

EFFECT OF ARTIFICIAL HAILING AND BLADE REMOVAL ON QUALITY OF GRAIN

The quality of the grain also was modified in these tests by the removal of all blades. Table III contains data on the percentage reduction in test weight of grain associated with the removal of all blades at different stages of growth.

The greatest decrease in test weight occurred when the plants were

TABLE III

REDUCTION IN TEST WEIGHT OF GRAIN PRODUCED BY PLANTS FROM WHICH ALL BLADES WERE REMOVED AT DIFFERENT STAGES OF DEVELOPMENT

STAGE	REDUCTION OF TEST WEIGHT IN		
	1928	1929	AVERAGE
	%	%	%
Vegetative (plants 4 feet tall)		7.8	7.8
Tassel emerging		No grain	
Ear shoot emerging		No grain	
Fresh silk		17.6	17.6
Early blister	15.0		15.0
Blister	22.8	23.3	23.1
Early milk	16.1		16.1
Late milk		14.5	14.5
Early dent	1.5	4.8	3.2

stripped of their leaves soon after the fertilization period. Blade removal when the plants were in the vegetative stage reduced yields markedly, but

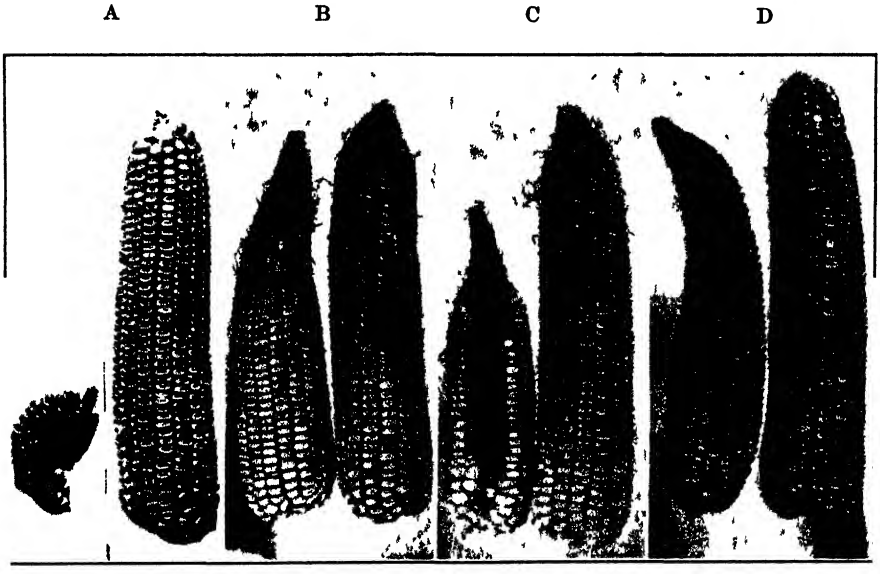


FIG. 5. Different types of adjustment in quantity of grain in response to reduced leaf area. Ears on left were from treated plants, those on right from untreated ones: *A*, cob size greatly reduced following blade removal in vegetative stage; *B*, sacrifice of kernel development at tip in favor of kernels at middle and butt of ear; *C*, four rows of kernels abortive following removal of eight blades at silking time; *D*, general underdevelopment of all kernels as a result of removing all blades when grain was in blister stage.

the degree to which the test weight was lowered did not correspond to the yield reduction, owing to the ability of the less mature plants to adjust the size of ear to the greatly reduced photosynthetic area of the plant. This adjustment was brought about in three different ways. When the blades were removed before or just as the shoot emerged, the size of the cob was greatly reduced and only a comparatively few ovules and silks developed (fig. 5 *A*). When the ovules had been fertilized at the time of treatment, the immature blister-like kernels on the tip of the ear failed to receive further nourishment and therefore were sacrificed in favor of the kernels at the butt (fig. 5 *B*). Under some conditions, the kernels on the lower side of the ear as it hangs on the stalk failed to develop. Reduction of leaf area tended to weaken the shank so that the tip of the ear hung downward, thus inadequately exposing the silks on the under side of the ear to pollen. This resulted in an ear with rows of kernels missing owing to lack of fertilization (fig. 5 *C*). When all blades were removed, further development of the kernels was seriously checked. The ear on the left in figure 5 *D* was produced on a plant that had all blades clipped off when the grain was in the blister stage. Obviously such almost complete cessation of the filling process would have a great effect upon the quality as well as upon the quantity of the grain.

The bruising action of hailstones markedly affected quality of grain. In an experiment to determine the extent of injury from bruising the ears, corn plants were beaten with a whip made of a bundle of baling wire. This implement tore the blades, broke many midribs, and battered the partially developed ears. The data obtained from treating plants in this way at different stages of their development are presented in table IV.

TABLE IV

INFLUENCE OF ARTIFICIAL HAILING WITH A WIRE WHIP UPON PERCENTAGE INCREASE OF ROTTED EARS AND PERCENTAGE DECREASE IN BOTH TEST WEIGHT AND YIELD OF SHELLED CORN, 1927

STAGE	INCREASE IN NUMBER OF ROTTED EARS	REDUCTION IN	
		TEST WEIGHT	YIELD OF GRAIN
	%	%	%
Blister to early milk	118.5	7.27	30.55
Late milk	0	3.87	27.70
Glaze	0	1.47	6.18
Early dent	0	0	2.54

The number of rotted ears was more than doubled by the artificial hailing when the grain was between the blister and early milk stages.

There was no increase in percentage of rotted ears for treatments applied in the late milk or later stages. The test weight per bushel and the yield of grain were decreased by treatments administered later in the plant's development. This decrease was due in large measure to the destruction of the leaf area rather than to the bruising effect of the wire whip on the ears.

Influence of leaf area removed on yield

In estimating the reduction in corn yield resulting from blade removal, it is as important to know the influence of the percentage of leaves destroyed as it is to know the effect of the stage of plant development. Data were therefore obtained on this phase of the problem by removing different percentages of the plant's leaves at various stages of growth (table V and fig. 6).

TABLE V

Reduction in yield of corn as a result of removing different percentages of leaf area at various stages of development, 1929

Blades removed	Reduction in yield of shelled corn as a result of removing blades				
	In tassel with shoot just emerging (July 31)	Fresh silks showing (Aug. 9)	Early blister stage (Aug. 16)	Milk stage (Aug. 30)	Early dent stage (Sept. 12)
%	%	%	%	%	%
8.3	*5.66**	9.00	4.93	5.54	+3.52
16.7	5.18	*12.47*	9.26	*12.98*	+4.13
25.0	*26.19*	*12.14*	*13.47*	*13.37*	7.82
33.3	*28.25*	*24.98*	*26.41*	*17.37*	6.55
50.0	*37.34*	*36.28*	*33.77*	*28.45*	*16.97*
66.7	*49.16*	*52.12*	*44.76*	*21.84*	9.51
83.3		*69.99*	*61.15*	*36.86*	*16.83*
100.0	*100.00*	*98.61*	*76.55*	*50.18*	*10.14*

* Italicized data were accompanied by odds of greater than 30:1.

A reduction in yield resulted when as small a leaf area as 8.3 per cent., which represents one average leaf per plant, was removed. The severity of the injury was roughly in proportion to the number of leaves removed. Even though the data are irregular it may be concluded that the stage of plant development, with the exception of the early dent stage, had little influence upon the yield reduction up to and including the removal of 25 per cent. of the leaves. Losses in the tassel, fresh silk, and early blister stages ran closely together up to the removal of 66.7 per cent. of the blades.

The curves of reduction in grain yield show a slight tendency to be

of an exponential type. Probably the efficiency of the remaining leaves is increased by the removal of blades, thus preventing the grain yields from falling off in direct proportion to the amount of leaf area destroyed. On the other hand, a certain rather constant amount of energy is required to maintain the life of the plant. When this is deducted from that supplied by the products of photosynthesis, there is only a limited quantity

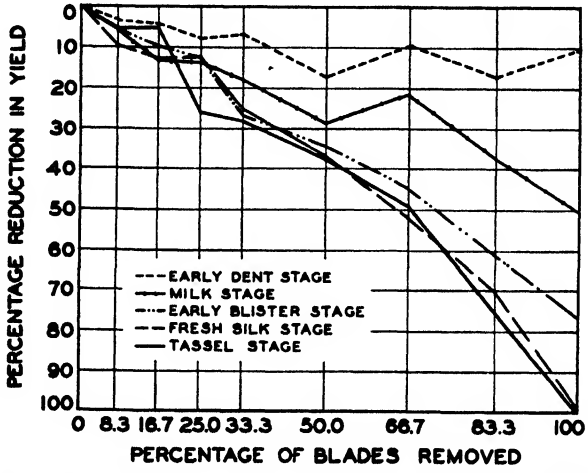


FIG. 6. Percentage reduction of grain yield following removal of different percentages of leaf area of corn plants in different stages of development.

left with which to form grain. With a decreasing supply of carbohydrates available as an increasing number of blades are cut away, the amount remaining to form grain would be disproportionately reduced. Thus the increased efficiency of the remaining blades tending to hold the yield up, and the constant maintenance demand exerting a drain on the elaborated food to the very last, result in a graph of yield reduction that resembles an exponential curve.

INFLUENCE OF REMOVING DIFFERENT PARTS OF BLADES

Since hail riddles the tips of blades badly and usually breaks off pieces of the outer portions, a test was made to determine whether there is any difference in the efficiency of the different parts of the blades. Table VI shows the reduction in yield following the removal of one-half the blade area in three different ways. The outer half of each blade was cut off, one side of the lamina of each blade was torn away, and six average blades were removed. The tests in 1928 included cutting out sections 4 inches long on alternate sides of the midrib. Because of the ease with which the

TABLE VI

COMPARISON OF YIELD REDUCTION ASSOCIATED WITH REMOVAL OF ONE-HALF THE BLADE SURFACE IN DIFFERENT WAYS AT VARIOUS STAGES OF DEVELOPMENT, 1929

Stage	Date	Reduction in yield					
		Outer half of each blade removed		One side of each blade removed		Six average blades removed	
		Percentage	Odds	Percentage	Odds	Percentage	Odds
		%		%		%	
Ear shoots emerging	July 31	23.93	>9999:1	38.26	>9999:1	37.34	>9999:1
Silks fresh	Aug. 9	34.79	>9999:1	38.55	>9999:1	36.44	>9999:1
Early blister	Aug. 16	32.51	>9999:1	34.66	>9999:1	33.77	>9999:1
Milk	Aug. 30	24.11	>9999.1	29.39	>9999:1	28.45	>9999:1
Early dent	Sept. 12	4.15	4:1	8.30	9:1	16.97	1110:1

remaining 4-inch sections were torn off by the wind, this method of removing half the leaf area was not used in 1929.

In the main the yield reduction was practically the same for all three methods of taking off half the leaf area, as shown by the data in table VI. There seemed, however, to be a tendency for the removal of the outer half of each blade to be less injurious than the tearing away of one side of each blade or the cutting away of half the number of leaves. Since the tests conducted the year previous (5) with a different variety of corn showed the removal of the tip half of the blades to be slightly more severe than the other methods of removing half the leaf area, it is believed that less than half of the blade area was removed in the 1929 experiment. The blades of the variety designated F_1365, used in 1929, were very wide at the base and it would be easy to make this error. At any rate it seems justifiable to conclude that it is mainly the percentage of blade area destroyed at any one time that influences the yield, rather than the particular portion of the blade that is destroyed.

One of the most common types of injury resulting from hailstorms is the shredding of the blades and the tearing of the laminae of the leaves from the midrib. In order to see how harmful this type of mutilation is, plants were treated by stripping the sides of the blades from the midrib but leaving them attached to the plants at the base (fig. 3 *C*). The data secured from applying this treatment to plants in different stages of development are presented in table VII.

The maximum injury was a little over 25 per cent. Since the reduction in yield for removing all the blades in the tassel-emerging stage was 100 per cent., it appears that the blades torn from the midrib are about

TABLE VII

YIELD REDUCTION WHEN BLADES WERE STRIPPED FROM MIDRIB BUT LEFT ATTACHED TO BASE, 1929

STAGE	DATE	REDUCTION IN YIELD	ODDS
		%	
Vegetative	July 20	15.91	2499:1
Tassel emerging	July 25	25.73	>9999:1
Ear shoots emerging	July 31	25.02	>9999:1
Silks fresh	Aug. 9	22.41	>9999:1
Early blister	Aug. 16	20.47	>9999:1
Milk	Aug. 30	11.97	28:1
Early dent	Sept. 12	2.41	2:1

75 per cent. as efficient as normal ones. HUME and FRANZKE (7) found that splitting the tips of the blades produced an average reduction in yield of 12.2 per cent. when the plants were in the hail-critical stage. Splitting the blades before and after this stage produced smaller reductions in yield.

TABLE VIII

REDUCTION IN YIELD OF GRAIN RESULTING FROM SLIGHT BLADE INJURIES TO CORN PLANTS IN DIFFERENT STAGES OF DEVELOPMENT, 1929

STAGE	DATE	REDUCTION IN YIELD OF GRAIN			
		MIDRIB BROKEN 3 INCHES FROM STALK		BOTH SIDES OF BLADE CUT TO MIDRIB 3 INCHES FROM STALK	
		PERCENTAGE	ODDS	PERCENTAGE	ODDS
		%		%	
Vegetative plants, 4 feet tall	July 20	15.20	475:1	.....	.....
Ear shoots emerging	July 31	5.00	3:1	18.83	434:1
Silks fresh	Aug. 9	11.65	46:1	39.28	>9999:1
Blister	Aug. 16	18.24	3332:1	26.22	>9999:1
Milk	Aug. 30	14.57	434:1	25.76	>9999:1
Early dent	Sept. 12	3.83	3:1	6.95	9:1

EFFECT OF BREAKING MIDRIB AND CUTTING LAMINA

Strong winds often cause the supporting tissues in the midrib to collapse and a hailstorm causes much of this type of injury. In order to test the harmfulness of such breaks in the vascular system, midribs were broken artificially about 3 inches from the base of the blade. As a companion experiment, the laminae were cut to the midrib on each side about 3 inches

from the stalk, making it necessary that all translocative materials pass through the vascular elements of the midrib. The results of applying such treatments at different stages of the plant's development are shown in table VIII.

Cutting the blade to the midrib on each side was associated with a greater reduction in yield than breaking the midrib. The maximum reduction in yield was 39 per cent. for cutting the blades to the midrib and 18 per cent. for breaking the midrib. The results secured in 1928 (5) showed a 23 per cent. reduction as a maximum following the breaking of the midrib and a 20 per cent. maximum for cutting the blade to the midrib. The difference is due to the stronger wind of 1929, which caused the breaking of the midrib of many of the blades that had been cut to this point. These tests show that translocation can take place through the lamina alone or through the midrib alone, without being accompanied with severe reductions in yield. So long as a blade part is attached to the plant, it seemingly possesses the ability to function photosynthetically and to translocate elaborated reserves to the ear.

Blade removal and breaking of ear shanks

A noticeable effect of blade removal was a reduction in the strength of the ear-supporting shank. This was especially apparent when the blades were removed during the earlier stages of the plant's development. A record of the percentage of shanks that were broken in the 1928 tests is presented in table IX.

Since the breaking of shanks is influenced both by the size of the ear and by the strength of supporting tissue, extremely early blade removal would be expected to result in a low percentage of broken shanks because the ears were too small to break them over. When the blades were removed after the ears had developed partially, the weight was sufficient to overtax the strength of the shanks. It is evident that the strengthening fibers of the shanks reach their final development rather late in the growing period, for blade removal when the grain was in the early dent stage resulted in no increase in percentage of broken shanks.

Estimating losses from hail

The problem of estimating losses resulting from hail injury involves a number of considerations. It is difficult to adjust for deteriorations in quality of grain except for the increase in percentage of rotted ears. This can be judged only by husking and examining the ears a considerable time after the storm. It probably is not desirable to attempt to ascertain the loss in quality as expressed by test weight per bushel. Feeding experiments reported by Rusk and Snapp (10) indicate that the dry matter in

TABLE IX

PERCENTAGE OF EAR SHANKS FOUND BROKEN AT HARVEST TIME ON PLANTS FROM WHICH A DIFFERENT NUMBER OF BLADES HAD BEEN REMOVED AT VARIOUS GROWTH STAGES, 1928

NUMBER OF BLADES REMOVED PER PLANT	PERCENTAGE OF SHANKS BROKEN WHEN TREATED ON				AVERAGE OF AUG. 11, 18, AND 28 TREATMENTS
	AUG. 11	AUG. 18	AUG. 28	SEPT. 15	
	%	%	%	%	%
1	51.3	47.2	50.0	61.1	49.5
2	63.2	66.7	70.0	63.9	66.6
3	68.3	71.4	61.5	59.5	67.1
4	78.9	69.4	71.8	58.3	73.4
5	75.7	65.0	61.5	51.4	67.4
6	90.9	76.3	82.9	51.3	83.4
7	82.0	83.3	76.9	64.9	80.7
8	90.0	77.8	80.0	31.2	82.6
9	77.5	83.8	80.0	54.3	80.4
10	81.1	84.2	85.7		83.7
11	92.5	94.9	83.8		90.4
All	91.7	100.0	77.1	64.9	89.9

well preserved, immature corn has practically as high a nutritive value, pound for pound, as the dry matter in sound, mature corn.

Natural hailstorms inflict injury of so many types and degrees of severity that it is next to impossible to duplicate them in experimental tests. The following types of damage may occur in combination on the same plant: (1) loss of blades or portions of blades, (2) shredding of the leaf portions remaining on the stalk, (3) part or all of the midribs broken, and (4) stalks and developing ears more or less severely bruised. Add to this the fact that the severity of natural hail usually varies in different parts of even a small field and the problem of correctly determining the loss appears difficult indeed.

Results of these investigations offer some assistance, however, in that they show the percentage loss in yield that may be expected to follow the removal of a given percentage of the leaf area. Furthermore, they show rather definitely the stage of development at which the corn plant is most susceptible to injury.

The data show that of the factors studied the destruction of blades exerts the greatest influence in the direction of yield reduction. Shredding the laminae from the midrib, even though every blade be so treated, produced a maximum yield reduction of only 25 per cent.

The work of ascertaining the amount of leaf area destroyed may be

done several days or even weeks after the storm, but notes on the stage of the corn development should be carefully taken immediately after the storm has subsided, for data clearly show that the stage of growth influences the severity of the losses as much as does the percentage of the leaf area destroyed.

Summary

1. Experiments covering a period of five years have been conducted with corn in an effort to determine the influence of blade removal, as well as other leaf and plant injuries of the types caused by hail, upon the quality and quantity of grain produced.

2. Although a definite manual of directions for hail insurance adjustment cannot be written at this time, certain fundamental principles are established through these experiments which may well serve as an aid to one's judgment in estimating hail injury.

3. It was found that the critical stage for leaf injury is at the time the ear shoots are just emerging. Leaf removal and injury before or after this stage is progressively less harmful to yields, becoming very slight in the dent stage.

4. The quality of grain as indicated by test weight per bushel was reduced most by leaf removal when the developing grains were in the blister stage. On the other hand, rotting of ears following bruising action of artificial hailing was worst when the treatment was applied during the early milk stage.

5. Tearing the sides of the blades from the midribs but leaving them intact at the base resulted in a yield 75 per cent. higher than complete removal of the blades. This indicates that as long as a leaf blade or part of it is attached to the plant, it is capable of functioning.

6. Reductions in yield at all growth stages correspond roughly with the percentage of leaf area removed, suggesting the possibility of estimating the loss from hailstorms from a determination of the leaf surface destroyed. Suggestions are made for ascertaining losses from hail, using data compiled in these experiments.

DEPARTMENT OF AGRONOMY
ILLINOIS AGRICULTURAL EXPERIMENT STATION
URBANA, ILLINOIS

LITERATURE CITED

1. CONNELL, J. H. Corn fodder. Mississippi Agr. Exp. Sta. Ann. Rpt. 3. p. 26. 1890.
2. CULPEPPER, C. W., and MAGOON, C. A. Effects of defoliation and root pruning on the chemical composition of sweet corn kernels. Jour. Agr. Res. **40**: 575–583. 1930.

3. DIBBLE, C. B., and MARSTON, A. R. Topping corn does not control corn borer. Mich. Agr. Exp. Sta. Quart. Bull. **11**: 195–197. 1929.
4. DUNGAN, G. H. Effect of hail injury on the development of the corn plant. Jour. Amer. Soc. Agron. **20**: 51–54. 1928.
5. ————————. Relation of blade injury to the yielding ability of corn plants. Jour. Amer. Soc. Agron. **22**: 164–170. 1930.
6. ELDREDGE, J. C. How hail storms damage corn. Wallace's Farmer **55**: 1296–1297. 1930.
7. HUME, A. N., and FRANZKE, CLIFFORD. The effect of certain injuries to leaves of corn plants upon weights of grain produced. Jour. Amer. Soc. Agron. **21**: 1156–1164. 1929.
8. HUNT, T. F. Corn topping *vs.* cutting whole stalks or allowing corn to ripen without cutting. Pennsylvania Agr. Exp. Sta. Rpt. 15. pp. 58–60. 1891.
9. NEWMAN, W. H. Experiments with corn. Alabama Canebrake Agr. Exp. Sta. Bull. 10. p. 8. 1890.
10. RUSK, H. P., and SNAPP, R. R. The utilization of soft corn in beef cattle feeding. Illinois Agr. Exp. Sta. Bull. 313. pp. 1–28. 1928.
11. SAYRE, J. D., MORRIS, V. H., and RICHEY, F. D. The effect of preventing fruiting and of reducing the leaf area on the accumulation of sugars in the corn stem. Jour. Amer. Soc. Agron. **23**: 751–753. 1931.
12. STEGGERDA, MORRIS. Effect of somatic injury upon yield in corn. Plant Physiol. **5**: 432–435. 1930.
13. TRACY, S. M., and LLOYD, E. R. Corn cutting, topping and stripping. Mississippi Agr. Exp. Sta. Bull. 33. pp. 63–64. 1895.
14. U. S. Dept. Agr. Percentage reduction from full yield per acre of 8 crops from stated causes, 1909–1924. Crops and Markets **3**: (Supplement 1, 11, 12). 1926.
15. VALGREN, V. N. Crop insurance: risks, losses, and principles of protection. U. S. Dept. Agr. Bull. 1043. pp. 1–27. 1922.
16. WEIGERT, J. Hagelschaden an unseren Kulturpflanzen. Landw. Jahrb. Bayern **3**: 49–57. 1913.
17. WYLIE, R. B. Cicatrization of foliage leaves. I. Wound responses of certain mesophytic leaves. Bot. Gaz. **90**: 260–278. 1930.

FACTORS IN ELONGATION AND EXPANSION UNDER REDUCED LIGHT INTENSITY

FREDERIC E. CLEMENTS AND FRANCES L. LONG

(WITH FOUR FIGURES)

In seeking to determine the rôle of factor and function in adaptation in nature, the first step has been to deal under control with one factor, and the function directly concerned, by equalizing the others. As an outcome of the results thus secured, it has been possible to modify the method to take into account two variables, such as light and water, in the hope of evaluating their respective rôles. This is particularly desirable in the case of adaptation to shade, in which reduced light intensity has generally been regarded as the controlling agent, with little or no consideration of water relations. Since water serves both as a raw material and as a mechanical force in growth, it would seem on theoretical grounds to play a significant part in such modification. This assumption has been borne out by the behavior of ecads in nature and especially by that of transplants in shade at times and seasons when the water content was deficient. The present account deals with the investigation of the mutual relation of light and water as exhibited by growth and modification in a series of lath-houses, which have been found to afford better control than cloth tents.

Experimental technique

The standard phytometer method was employed, in which are utilized galvanized iron containers 8.5 inches wide and 11 high, provided with removable lids containing a 2-inch hole to accommodate the stem and a watering tube. A layer of coarse gravel an inch thick was placed in the bottom of the can and the weight of the latter then adjusted to a total of 2000 gm. by varying the gravel. Air-dry loam to the amount of 6600 gm. was added, together with 132 gm. of water or 20 per cent. of the dry weight, and the containers left over night to permit even penetration. In filling each can, the glass tube 1.5 cm. across was held against the bottom to prevent clogging and was raised a half centimeter at the first watering to allow ready absorption.

After soaking the sunflower seeds over night, five were placed near the center of the container and allowed to germinate in the greenhouse. By two weeks after planting, the first pair of true leaves were well developed, and all but one of the seedlings in each can were pulled out. In this operation much care was taken to select individuals for uniformity in height and the size of the leaves.

The essential feature of the experimental procedure was to vary the holard under each of several degrees of light intensity, though the installation was improved in certain details through the five series. The first two sets were preliminary in nature and only the two extremes of holard, namely, 35 and 13 per cent., were used. In the remaining series, there were four different water contents and either three or four effective intensities of light. The technique is best illustrated by series V in which 96 phytometers were utilized. These were organized into four batteries of 24 each, to three of which water was added by weight to bring the holard to 35, 26, and 18 per cent. of the dry weight. Since the soil used is saturated at 42 per cent., the values represented respectively 83, 62, and 43 per cent. of saturation. No water was added to the fourth battery, which was allowed to dry out until the water content reached 13 per cent. or 31 per cent. of saturation. A layer of 500 gm. of sand was employed to prevent evaporation but at the same time to permit adequate aeration. Finally, the lid was put on the can, the glass-tube stoppered, and a seal of non-absorbent cotton wrapped about the stem in such manner as to completely close the opening.

In this series the light gradient comprised four intensities, *viz.*, full sunlight, 32, 16, and 8 per cent. of sunlight as afforded by the group of lath-houses. A battery of 24 phytometers was installed in each of the latter, as well as in the sun, where the containers were placed in a wooden box sunken in the soil to prevent overheating of the walls and consequent interference with root functions. As indicated previously, each battery embraced four different holards, represented by six phytometers each. The higher value of 35 per cent. was the maximum for the soil, since an occasional effect of deficient aeration was found in the lower light intensities, while the extreme of 13 per cent. now and then permitted slight wilting under high insolation. A strain of *Helianthus annuus* with simple stems was used in all series, and this was supplemented in the last one by *Clarkia elegans*, a California annual with exceptional adaptability to shade.

Though it was impossible to equalize temperature and humidity in the garden and the lath-houses, the difference in average daily maximum temperature was but 1.5° F. and between average daily minima 4° F. throughout the spring period. There were only 3 degrees' difference in average temperature, this being highest in the lath-house of 16 per cent. intensity and the same in the sun and the lath-house with 8 per cent. The average humidity varied somewhat more, being nearly uniform in the lath-houses but rising several degrees in the sunlight, probably owing to the sea breeze. While the alternation of factor changes is more regular in lath-house than in forest, it is evident that it is of the same nature as that between sun-fleck and shadow below such a canopy.

The water content was kept practically constant by weighing the con-

tainers from time to time and replacing the amount of water lost. Frequent weighings were not necessary during the first few weeks while the plants were small, but as they grew larger, replacement was required more often. During the last week when temperatures were high, some individuals lost more than a liter of water per day in the sun and thus demanded weighing and watering at two-hour intervals to maintain the proper holard. In addition to the water requirement, the rate of growth in stem and leaf was traced week by week, and the wet and dry weights of each plant were ascertained at the close of each series. Measurements of stem height and diameter and of the length and width of each leaf were made each week at the same hour and in the same sequence. Leaf areas were calculated by the formula $L \times W \times 1.3$, which gives the combined area of the two sides (CLEMENTS and GOLDSMITH, "The Phytometer Method," 1924: 35); this method was again verified for the strain of sunflowers employed.

Results from series V

This was not only the most complete of the various series, but it also coincided with the time of the year when the adaptation sequences were at their optimum in the Santa Barbara climate, namely, during April and May. Series IV occupied the period of January to April, and number III that from October to December. The criteria employed were height and diameter of stem, number and area of leaves, wet and dry weights of the whole plant, water requirement and transpiration. For the sake of ready comparison, the data are presented in two sequences, *viz.*, arranged under the four holards in accordance with light intensities, and in the four light values by the percentage of holard.

With respect to the growth of the stem, the results were essentially consistent for diameter, the maximum occurring in 35 per cent. holard and sunlight and falling off rather regularly with reduced light. A slight discrepancy is found in sunshine for 18 per cent. and 13 per cent., owing to the fact that the rate of supply occasionally lagged behind water loss. The response in height was less consistent only to the extent that the maximum alternated between 16 per cent. and 32 per cent. light. On the other hand, the integration of stem growth as represented by height diameter (H/D) yielded a regular rise with decreasing light, only the extreme condition of 13 per cent. holard and 8 per cent. light being out of accord. Leaf area diminished with all but perfect regularity in harmony with light values, dropping very rapidly in the two lowest intensities, while there was no exception to the regular decrease in the number of leaves.

As was anticipated, functions in general and the relatively simple transpiration in particular exhibited the closest correspondence with factor gradients. Water loss was uniformly highest in the sun and fell off sharply

in light of 32 per cent. However, the greatest decrease was between 32 per cent. and 16 per cent., where it was threefold, or even sevenfold in 13 per cent. holard. The loss per cm.^{2} agreed with the total except for the lower three water contents in the 8 per cent. lath-house, a discrepancy probably associated with the small leaf area, crowded stomata, and thinner cuticle. In agreement with transpiration, both wet and dry weights dropped with light intensity, but the dry weight diminished more rapidly, the maximum being respectively 23, 23, 12, and 15 times the minimum in each holard, by contrast with 13, 12, 6, and 8 for the wet. On the other hand, the water

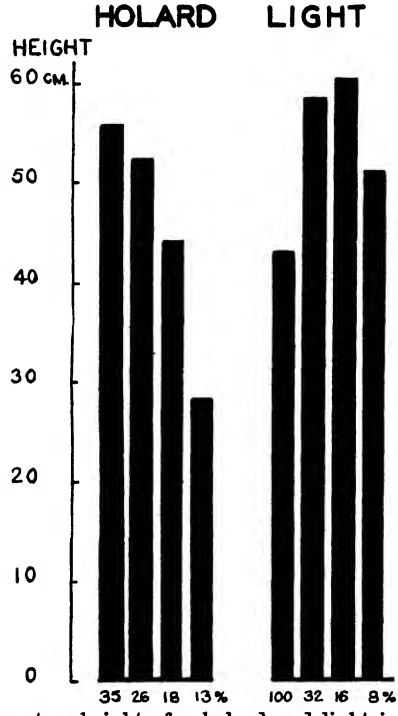


FIG. 1. Average stem heights for holard and light in the three series.

requirement rose steadily with weaker light, with the exception of the 13 per cent. holard, and this is to be explained by the fact that food-making decreased faster than water loss as the energy fell off, as shown by ratios of 23, 23, and 12 in the former, and 15, 11, and 7 in the latter.

Turning to gradients of holard under each of the four light intensities, stems were shorter in response to lower water values, with two exceptions, while diameters lessened in every case. Leaf areas followed the rule with but two insignificant discrepancies, and the number of leaves decreased throughout. Total transpiration is similarly in accord with the holard, as

is likewise the wet weight. Dry weight is slightly less harmonious, lack of aeration becoming perceptible in 35 per cent. holard, while the water requirement is all but entirely consistent.

Results from series III and IV

In addition to *Helianthus,* series IV contained *Clarkia elegans,* a native annual especially tolerant of shade. The holard gradient was the same, but the reduced light intensities were but two, namely, 16 per cent. and 8 per cent. As a consequence, the tallest stems were always found in the 16 per cent. value, while as before the stature was distinctly greater in 8 per cent. than in sunshine. The diameter of stem diminished consistently with the

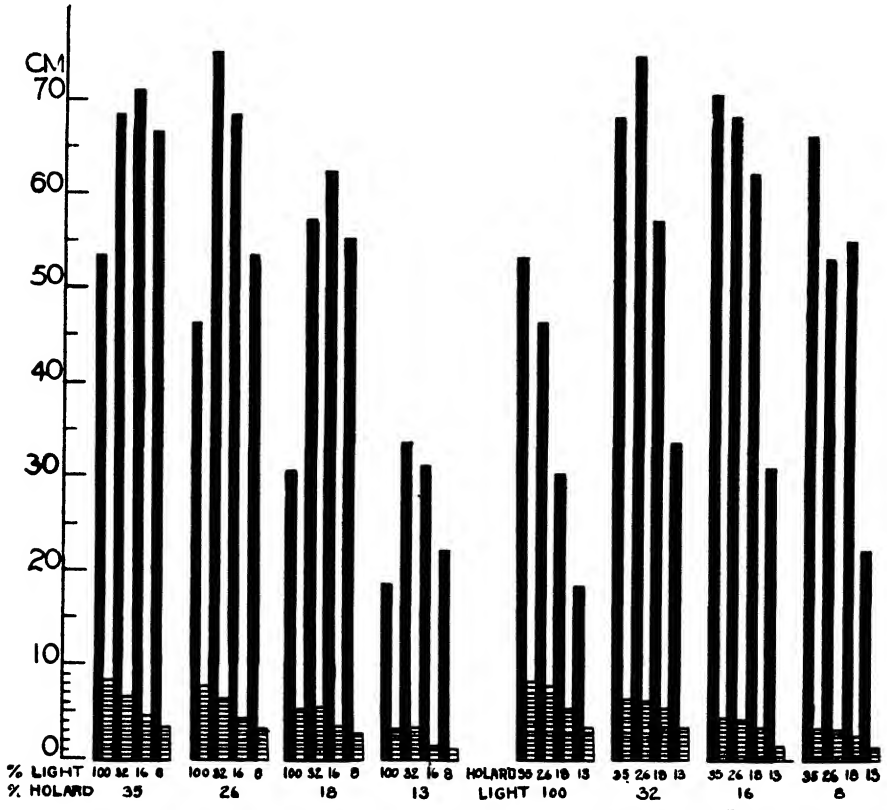


FIG. 2. Stem height (×1) and diameter (×5) in series V.

three intensities, as did also leaf area, number of leaves, wet and dry weights; conversely, height/diameter of stem was enhanced. Total transpiration fell off in entire harmony with light reduction, as did likewise the water loss per cm.,[2] with the exception of a slight rise in 8 per cent. under

13 per cent. holard. By contrast, the water requirement rose regularly except for a small discrepancy in 8 per cent. light and 26 per cent. water content. The values for the holards within each light intensity were less consistent, the plants in 26 per cent. usually growing better than those in 35 per cent. This discrepancy seems to be explained by the fact that this

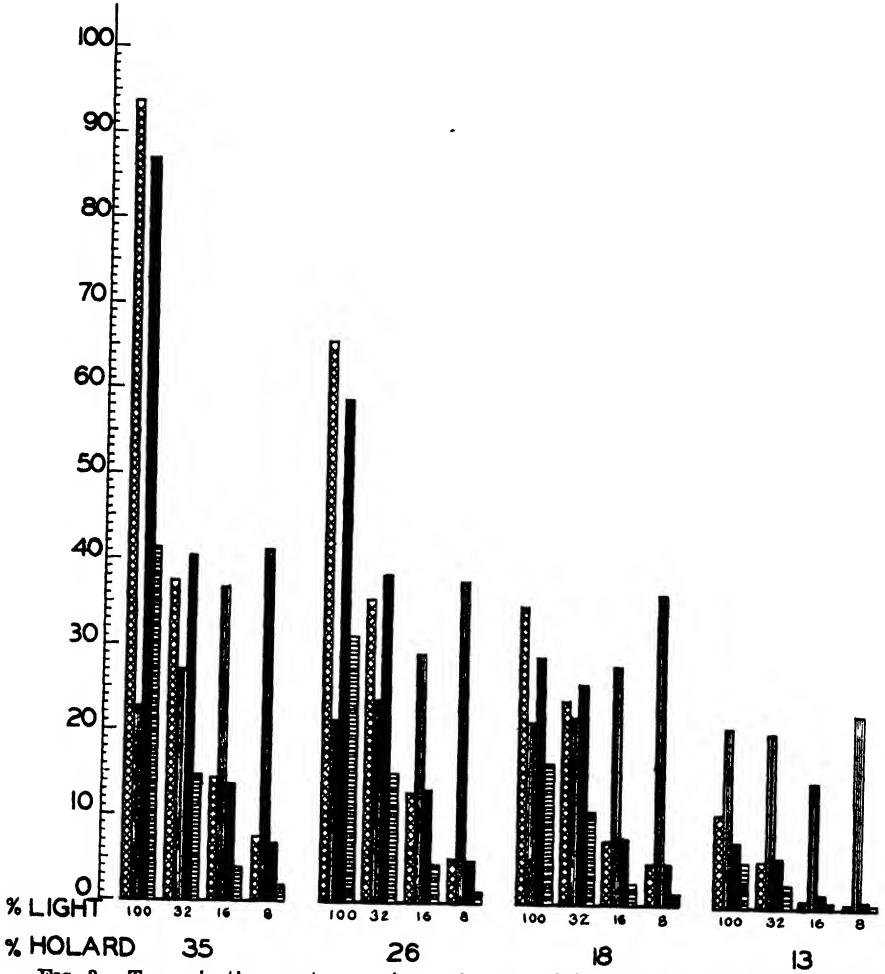


FIG. 3. Transpiration, water requirement, wet weight, and dry weight in sequence for series V, the basing unit representing 100 cc. transpiration per plant, 10 cc./gm. water requirement, 4 gm. wet wt. and 1 gm. dry wt.

series was begun in January when transpiration did not compensate for the high water content and the resulting tendency toward deficient aeration.

The relations of stem and leaf to the factor gradients were distinctly less uniform for *Clarkia*. Wet and dry weights dropped consistently with

both light and holard, as did likewise the total transpiration, but maximum stature and leaf size alternated between sun and 16 per cent. light. Stem diameter was more regular in response, but water requirement was quite erratic. The divergence in behavior as compared with the sunflower is probably to be ascribed to the necessity of transplanting the individuals as seedlings, thus favoring the production of lateral shoots to the disadvantage of the main stem.

Series III represented the original installation that was duplicated in IV, and was grown from October to December of the preceding autumn. The two corresponded almost exactly in the trend of results, except that water requirement in the earlier set was uniformly higher in 16 per cent. light.

For comparison with the response in the lath-houses where the light reduction was produced by a moving shadow, sunflower batteries were placed under light arcs approximately 10 ft. at the base, 6 ft. high and 3 ft. wide. One of these was covered with cheesecloth, the other with paper, yielding respective light values of 62 and 14 per cent., by contrast with full sunshine. For the gradient, the values were the most variable obtained during the entire course of the experiment. Height of stem, leaf area and number, wet weight, and water requirement were not concordant in the four holards, nor with the results in other series. However, diameter gave a positive correlation with decreasing radiant energy, as did dry weight and total transpiration, a single marked exception occurring in 13 per cent. holard in the sun, where wilting disturbed the relation. The values for sunlight and 62 per cent. light were regularly much greater than for 14 per cent., as would be expected, but they were also much closer to each other and alternated more frequently than the difference in intensity would warrant on the basis of a direct relation. Aside from water requirement, the values for the holard sequence in each light intensity were essentially consistent throughout, except for a slight decrease in the 35 per cent. in both degrees of shade, due it would seem, to deficient aeration.

In order to test the rôle of water in combination with another factor, a third subseries of the same four holards was grown under three wind conditions, namely, 0, 5, and 10 miles per hour. Apart from the 5-mile wind under 13 per cent. water content, the results as to growth and water requirement were practically all consistent. Stem height and width gave a negative correlation with increasing velocity, the two being in harmony with each other instead of opposed, as in the case of light. The leaf area and number also fell off as the wind velocity rose, as did wet and dry weight, while the water requirement was augmented. The total transpiration was highly variable, but the water loss per cm.^{2} rose with much regu-

TABLE I

RESULTS FROM SERIES V

FACTOR GRADIENTS		STEM					LEAF AREA PER PLANT		LEAVES	
		HEIGHT		DIAM.		H/D				
HOLARD	LIGHT									
%	%	cm.	%	cm.	%		sq. cm.	%	No.	%
35	100	53.3	100	1.66	100	32.1	4556.6	100.0	23.7	100
	32	68.3	128	1.30	78	52.5	3124.5	69.0	18.5	78
	16	70.9	133	0.91	55	72.8	1122.3	25.0	14.3	60
	8	66.3	125	0.68	41	97.5	648.6	14.0	12.5	53
	Ave.	64.7	122	1.14	69	63.7	2458.5	52.0	17.2	73
26	100	46.3	87	1.58	95	23.9	3420.0	75.0	22.2	94
	32	75.0	141	1.25	75	60.0	2641.5	58.0	18.0	76
	16	68.3	128	0.83	50	82.3	1195.5	26.0	13.2	56
	8	53.5	100	0.61	37	87.8	435.8	10.0	11.3	48
	Ave.	60.8	114	1.07	64	63.5	1923.2	42.0	16.2	69
18	100	30.5	57	1.05	63	29.1	1743.3	38.0	17.2	73
	32	57.3	108	1.08	65	53.0	1939.1	43.0	15.2	64
	16	62.5	117	0.71	43	88.0	727.2	16.0	12.3	52
	8	55.3	104	0.55	33	100.5	439.6	10.0	11.0	46
	Ave.	51.4	97	0.85	51	67.9	1212.3	27.0	13.9	59
13	100	18.8	35	0.65	39	28.9	521.5	11.4	12.2	51
	32	33.6	63	0.66	40	51.0	499.6	10.9	10.5	44
	16	31.0	58	0.28	17	111.0	114.2	2.5	8.2	35
	8	22.1	41	0.23	14	96.0	65.2	1.4	6.1	26
	Ave.	26.4	49	0.46	28	71.7	300.1	6.6	9.3	39

FACTOR GRADIENTS		TRANSPIRATION		WEIGHT OF PLANT			WATER REQUIREMENT	
		TOTAL	GM./ SQ. CM. MAY 7–14	WET	DRY	RELATIVE WEIGHT		
HOLARD	LIGHT							
%	%	cc.	gm.	gm.	gm.	%	cc./gm.	%
35	100	9359.0	134.5	347.7	41.23	100.0	227	100.0
	32	3727.0	80.9	162.1	13.70	33.0	272	120.0
	16	1428.0	78.5	54.2	3.88	9.4	368	162.0
	8	741.5	64.2	26.6	1.80	4.4	412	181.0
	Ave.	3563.9	89.5	147.7	15.15	36.7	320	141 0
26	100	6564.3	99.6	234.9	31.02	75.0	211	92.9
	32	3535.5	78.4	153.2	15.00	36.0	236	104.0
	16	1290.0	64.5	52.4	4.43	11.0	291	128.0
	8	508.1	68.5	19.1	1.35	3.3	376	165.6
	Ave.	2974.5	77.8	114.9	12.95	31.3	279	122.6
18	100	3647.0	103.0	115.7	16.35	40.0	212	93.4
	32	2383.6	70.5	102.7	10.95	27.0	218	96.0
	16	748.5	59.0	31.8	2.68	6.5	279	122.9
	8	485.1	61.1	19.5	1.33	3.2	365	160.8
	Ave.	1771.1	80.9	67.4	7.83	19.2	269	118.3
13	100	1057.0	114.7	30.0	5.08	12.3	208	91.6
	32	527.5	69.1	22.8	2.63	6.4	201	88.5
	16	83.8	52.2	5.9	0.58	1.4	145	63.9
	8	79.1	79.7	3.7	0.35	0.9	226	99.0
	Ave.	434.9	78.9	15.6	2.16	5.2	195	85.7

TABLE I—(*Continued*)

RESULTS FROM SERIES V

FACTOR GRADIENTS		STEM					LEAF AREA PER PLANT		LEAVES	
LIGHT	HOLARD	HEIGHT		DIAM.		H/D				
%	%	*cm.*	%	*cm.*	%		*sq. cm.*	%	*No.*	%
100	35	53.3	100	1.66	100	32.1	4556.6	100.0	23.7	100
	26	46.3	87	1.58	95	29.3	3420.0	75.0	22.2	94
	18	30.5	57	1.05	63	29.1	1743.3	38.0	17.2	73
	13	18.8	35	0.65	39	28.9	521.5	11.4	12.2	51
	Ave.	37.2	70	1.24	74	29.9	2560.3	56.1	18.8	80
32	35	68.3	128	1.30	78	52.5	3124.5	69.0	18.5	78
	26	75.0	141	1.25	75	60.0	2641.5	58.0	18.0	76
	18	57.3	108	1.08	65	53.0	1939.1	43.0	15.2	64
	13	33.6	63	0.66	40	51.0	499.6	10.9	10.5	44
	Ave.	58.5	110	1.08	65	54.1	2051.2	45.2	15.6	66
16	35	70.9	133	0.91	55	72.8	1122.3	25.0	14.3	60
	26	68.3	128	0.83	50	82.3	1195.5	26.0	13.2	56
	18	62.5	117	0.71	43	88.0	727.2	16.0	12.3	52
	13	31.0	58	0.28	17	111.0	114.2	2.5	8.2	35
	Ave.	58.2	109	0.68	41	88.5	789.8	17.4	12.0	51
8	35	66.3	125	0.68	41	97.5	648.6	14.0	12.5	53
	26	53.5	100	0.61	37	87.8	435.8	10.0	11.3	48
	18	55.3	104	0.55	33	100.5	439.6	10.0	11.0	46
	13	22.1	41	0.23	14	96.0	65.2	1.4	6.1	26
	Ave.	49.3	93	0.52	31	95.5	397.6	8.9	10.2	43

FACTOR GRADIENTS		TRANSPIRATION		WEIGHT OF PLANT			WATER REQUIREMENT	
LIGHT	HOLARD	TOTAL	GM./SQ. CM. MAY 7–14	WET	DRY	RELATIVE WEIGHT		
%	%	*cc.*	*gm.*	*gm.*	*gm.*	%	*cc./gm.*	%
100	35	9359.0	134.5	347.7	41.23	100.0	227	100.0
	26	6564.3	99.6	234.9	31.02	75.0	211	92.9
	18	3467.0	103.0	115.7	16.35	40.0	212	93.4
	13	1057.0	114.7	30.0	5.08	12.3	208	91.6
	Ave.	5111.8	113.0	182.1	23.42	56.8	215	94.5
32	35	3727.0	80.9	162.1	13.70	33.0	272	120.0
	26	3535.5	78.4	153.2	15.00	36.0	236	104.0
	18	2383.6	70.5	102.7	10.95	27.0	218	96.0
	13	527.5	69.1	22.8	2.63	64.0	208	88.5
	Ave.	2544.4	74.7	110.2	10.57	40.0	234	102.1
16	35	1428.0	78.5	54.2	3.88	9.4	368	162.0
	26	1290.0	64.5	52.4	4.43	11.0	291	128.0
	18	748.5	59.0	31.8	2.68	6.5	279	122.9
	13	83.8	52.2	5.9	0.58	1.4	145	63.9
	Ave.	887.6	63.6	46.0	2.89	7.1	271	119.2
8	35	741.5	64.2	26.6	1.80	4.4	412	181.0
	26	508.1	68.5	19.1	1.35	3.3	376	165.6
	18	485.1	61.1	19.5	1.35	3.2	365	160.8
	13	79.1	79.7	3.7	0.35	0.9	226	99.0
	Ave.	453.5	68.4	17.2	1.21	2.9	345	151.5

larity in the higher water contents, the relative values being 2, 3, 5 for 35 per cent. and 1, 2, 3 for 18 per cent.

With respect to the holard gradient under the several velocities, stem height and diameter diminished with reduced water values, and this was equally true of leaf area and number, as likewise of wet and dry weights. The same rule held for water requirement with a single exception, and applied consistently to the total water loss but less regularly to transpiration per unit of leaf area.

TABLE II

COMPARISON OF RESULTS FROM THE THREE SERIES

	HOLARD-LIGHT			LIGHT-HOLARD		
	V	IV	III	V	IV	III
Stem						
height	m16–32	m16	m16	−r	m26	−r
diam.	−r	−c	−r	−c	−r	−r
ht./diam.	+r	+c	+r	d	−d	−r
Leaf						
area	−r	−c	−c	−r	m26	−c
number	−c	−c	−c	−c	m26	−r
Weight						
wet	−c	−c	−c	−c	m26	−c
dry	−c	−c	−c	−r	m26	−r
Transpiration						
total	−c	−c	−c	−c	m26	−r
per cm.^{2}	−r	−r	−d	−d	−r	−d
Water require.	+r	+r	m16	−r	d	d

In the above table, positive correlation with the decreasing gradient of light or holard is shown by the minus, negative by the plus sign: *c*, denotes consistent, *r*, regular, and *d*, discrepant. Where the maximum is out of the sequence, it is indicated by *m* with a figure for the condition. The agreement between the three series is close in the case of light, especially in view of the fact that the sun's altitude was respectively that of spring, winter, and fall. The accord is distinctly less for holard, by reason of the divergence of the fourth series for the most part, but also, it would seem, because of the unavoidable variation of holard in the interval between two waterings.

When the results in stem height are averaged for the three series, they yield the following percentages in the order of descending holard, *viz.*, 100, 93, 77, and 50, while for decreasing light the values are 100, 136, 140, and 118. For stem diameter, the respective figures in the same sequence are

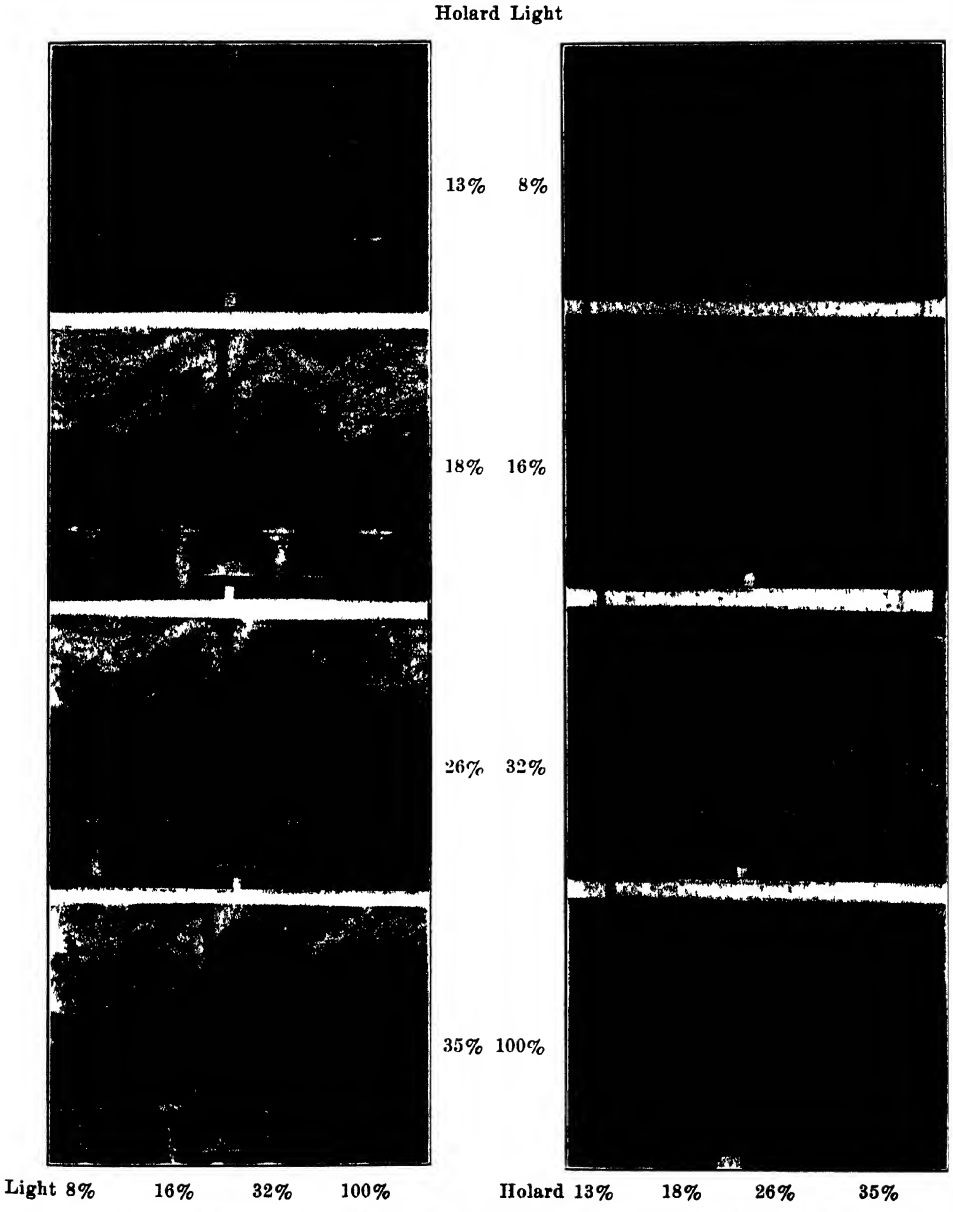


FIG. 4. A representative set from each battery of phytometers in series V, showing the growth under the various conditions.

100, 99, 78, and 58, and 100, 82, 61, and 49. Dry weight finds its maximum of 121 per cent. in 26 per cent. holard, falling then to 65 and 28, but for the light gradient it falls regularly and quite consistently from 100 to 45, 16 and 7 per cent. The grand averages for area and number of leaves drop with almost complete regularity for each factor, but while the five largest leaves for 35 per cent. holard are found in the sun, for all other water contents they occur in 32 per cent. light.

TABLE III

EXPANSION OF LARGEST LEAVES, SHOWN IN CM.²

HOLARD	LIGHT INTENSITY			
%	% 100	% 32	% 16	% 8
35	553.8	475.0	204.0	141.2
26	378.8	416.2	205.8	98.2
18	303.4	352.0	144.0	101.2
13	127.2	144.6	29.4	17.3

There is evidently a negative correlation between the expansion of the leaf and its thickness, but there are several difficulties in the way of the ready determination of the latter. These reside chiefly in the leaf itself and especially in the complication introduced by the veins. Variations in turgor are a further source of error, as is also the actual technique of measuring thickness on leaves in position. If allowance be made for shrinkage, the simplest method is to make measurements on microtome sections from representative areas on which to base a range or average. The histological material obtained from the various series is yet to be worked up in connection with similar adaptations in native transplants, but a preliminary study of leaf thickness has been made for series V. The averages for each holard gradient in the four light intensities are in fair correspondence with the latter, the thickness falling rapidly from sun to 32 and 16 per cent. and then slightly to 8 per cent., *viz.*, 0.2923, 0.2462, 0.2016, and 0.2002.

Relative rôles of water and light

In all the series, it is obvious that water plays a considerable part in the elongation of stems and the expansion of leaves. This is naturally to be expected, chiefly on the basis of its mechanical action in turgor, but to some extent also because of its use as raw material, and this is supported by the

responses in both native and control cultures. More difficult of answer, however, is the question as to the respective importance of water and light in growth under reduced light intensities. Probably the frequent statement that plants stretch up in the shade in order to secure more light is intended to be more or less figurative, but the response is so graphic as to seem to lend support to this explanation. The major objective of the present investigation is to discover whether the relation to each of the factors permits quantitative expression.

TABLE IV

MAXIMUM DIFFERENCES BETWEEN EXTREMES

LIGHT EFFECTS

HOLARD	STEM HEIGHT	DRY WEIGHT	LARGEST LEAVES
%	*cm.*	*gm.*	*cm.*^{2}
35	17.6	39.43	334
26	28.7	28.67	318
18	32.0	15.02	251
13	14.8	4.63	127
Average	23.3	22.19	257
LIGHT		**HOLARD EFFECTS**	
%	*cm.*	*gm.*	*cm.*^{2}
100	34.5	35.15	426
32	41.4	11.07	330
16	39.9	3.30	175
8	44.2	1.45	124
Average	40.0	12.74	264

The averages of the maximum differences for light and for water responses deviated little from each other in the case of the largest leaves, and this was true likewise of leaf area and stem diameter. However, both stem height and dry weight exhibited striking divergences. The maximum difference in stature produced by light within the various holards ranged from 14.8 to 32 cm., with an average of 23.3 cm., while for the holard gradient within each light set the respective values were 34.5 and 44.2 cm., with an average of 40 cm. Similarly, in the table for series V, the differences between the extreme averages for light intensities, namely, 37.2 and 58.5, is 21.3 cm., while for the four holards the corresponding figures are 64.7 and 26.4, with a difference of 38.3 cm. Hence, the excess of stature

in terms of response to water over that to light is 16.7 cm. in one case and 17 in the other. For all three series the difference between the greatest and least average height for the holard was 27.7 cm.; for light, it was 17.3 cm. On the basis of the average height for all series and conditions, the difference, 10.3 cm., amounted to 21 per cent. in favor of water.

With respect to the dry matter produced, the situation was reversed. The differences between extremes ranged from 4.63 to 39.43 gm. with an average of 22.19 gm. for light, to respective values of 1.45 to 35.15 gm. and an average of 12.74 gm. for water. For the three series, the difference between the highest and lowest averages was 12.33 gm. for water and 21.44 gm. for light. The excess to be ascribed to light is 8.9 gm., which approximates closely the average dry weight for all series and conditions, namely, 9.1 gm., and thus indicates that the rôle of light in producing construction material is twice as great as that of water. This relation is reflected in the production of photosynthate as measured by the picric acid method. From 8 per cent. light to full sunshine the amount rose from 0.384 to 1.408 mg. per cm.^{2}, a gain of 1.028 mg., while for the increase from 13 per cent. to 35 per cent. holard, the rise in sugars was but 0.45 mg. This was somewhat less than half as much, but the departure from the value derived from the dry weights is not serious.

From the foregoing, the conclusion seems warranted that variations in holard exert a minor effect upon the amount of material for the construction of stems while light plays the paramount rôle. On the other hand, the mechanical action of water is increased as the resistance offered by materials drops in proportion to photosynthesis. This would seem to find expression chiefly in the stem where the ratio between dry matter and sap-content is low by contrast with the leaf where the ratio is high. This is further indicated by the small differences in the averages for largest leaves and leaf areas (table IV). However, the results obtained during the past fifteen years in growing nearly a hundred species in lath-houses, length-of-day tents, in water and nutrient sequences, as well as a much larger number in transplant gardens, demonstrate that there may be considerable differences in behavior between species and genera as well as between life forms.

The major rôle assumed by the holard in the elongation of shoots serves to explain several apparent contradictions in the behavior of transplants, as well as of species subjected to factor gradients. At the outset it was expected that the transfer of alpine dwarfs from the summit of Pike's Peak to the plains at 6000 ft. or to the sea coast at Santa Barbara would produce an increase of stature. This did not happen in most cases and it was soon realized that this must be due to increased transpiration in the presence of a holard often tending toward a deficit. In short, the striking change of

climate had little or no effect upon elongation except during wet seasons, and to modify the dwarf habit effectively it became necessary to maintain an adequate water content. As to the prevalence of dwarfing in the alpine tundra itself, it had been earlier discovered that this was characteristic of climax and subclimax communities. Wherever disturbance reduced competition for water, as in the so-called "gopher gardens," or the supply was adequate as in wet meadows, the stature increased from two to fourfold, to equal that at much lower altitudes.

The controlling influence of water has also become more and more evident in natural shade gardens located in the spruce climax at 8000 ft. At the time of installation it was assumed that survival would be greater and modification more rapid than in transplanting into the sun, but the reverse often proved true, especially as to survival. This was found to be due to high interception of the frequent but light showers by the canopy and even more to the thick layer of needles and duff that decreased penetration to the root level. It was thought to cure this by sprinkling, but it finally became necessary to remove much of the duff to assure a proper water content. This accomplished, the shade gardens became the most prolific in striking modifications and pointed the way to the greater control and success achieved by means of lath-houses at the three altitudes.

Summary

Phytometer batteries of sunflowers have been employed in combinations of four degrees of holard with four of light to determine the respective rôles of these two factors in the apparent response to shade. The results obtained indicate that water assumes the major rôle in stem elongation, and light the larger part in the production of dry matter, the two necessarily cooperating in the adaptation characteristic of shady habitats.

THE ALPINE LABORATORY
CARNEGIE INSTITUTION OF WASHINGTON
MANITOU, COLORADO

THE COHESION THEORY OF TRANSPIRATION

ROBERT E. HUNGATE

(WITH TWO FIGURES)

The present explanation of the mechanism of water transport in plants (**11**, p. 9; **12**, p. 231) includes, briefly, the pulling of continuous columns of water from the soil by means of evaporation from the leaves, the columns remaining intact under the stress of great height and rapid evaporation because of the tensile strength of the water (**6, 16, 20**).

WALTER (**23**, p. 67), after a discussion of experiments on water transport, says, "Damit scheint die Theorie der Wasserleitung, die auf der Kontinuität der Wasserfäden von der Spitze bis zur Basis der Pflanzen beruht und deshalb als Kohäsionstheorie bezeichnet wird, endgultig gefestigt zu sein, wenn auch im Einzelnen immer noch Veränderungen eintreten können. . . ."

WOODHOUSE (**24**) reports experiments interpreted as indicating a need for modification of the tensile column theory. The experiments are: first, an inability, using *Pittosporum undulatum* and *Ricinus communis*, to draw mercury to a height greater than 49 cm., and, second, a greater speed of evaporation from a branch of *Ricinus* on a day with moderate conditions of evaporation than on a day of intense evaporation.

In the first case the inability to draw mercury to a height greater than 49 cm. can be explained as due to failure to clean and prepare the apparatus sufficiently and to remove air from the plant stem. BOEHM (**4, 5**) found that out of hundreds of experiments to demonstrate the rise of mercury above atmospheric pressure only a few were successful. With more refined methods THUT (**18**) and URSPRUNG (**21**) demonstrated the tensile strength of a column of water using living material. THUT (**17**) and OTIS (**13**) have described methods for its demonstration in a physical system. The experiments are sufficiently reliable to use in classroom exercises.

In the second case the difference in rate of evaporation of branches of castor bean on the warm and on the cool day can be ascribed to a probable difference in leaf area since this factor apparently was not evaluated. No arguments are presented to support the claim that the development of greater tension on a day of less evaporation makes the tensile column theory inadequate.

In addition WOODHOUSE interprets experiments with a 14-meter glass U-tube as throwing doubt on the tensile column theory. "The top of one arm of the U-tube was connected by a stopcock to a vacuum system. It was hoped that the velocity of the water, descending in one arm of the tube and

rising in the other, combined with its cohesive properties, would carry it to an elevation greater than the barometric column. Unlike ASKENASY (**3**) [**1**] and THUT (**102**) [**17**] under the conditions of this experiment no tensile column was obtained. The water entered the vacuum chamber in spurts, and numerous bubbles formed in the column."

In connection with this experiment it is important to consider the condition at the top of any column of water in which its cohesive properties are to be demonstrated. According to RENNER (**14**): "Die Höhe der als unzerreissbar angenommen Wassersäule, die in einer beliebig weiten Röhre an einer porösen Membran aufgehängt werden kann, hängt von der Porenweite dieser Membran ab. Die Wassersäule kann genau so hoch werden, wie das Wasser in einer Kapillarröhre steigen würde, deren Weite auf ihrer ganzen Länge gleich der Weite der gröbsten Poren der Membran ist."

In the experiment of WOODHOUSE the diameter at the top of the glass tube in contact with the gas in the vacuum chamber was 1.75 mm. The top of the water column was not attached in any way and could not be supported by its tensile strength. The height to which water rises in such a tube is determined by the difference in gas pressure between the base and top of the column and the force of surface tension at the top. The surface tension in a tube of the size used will support a column of water to a height of 18 mm. as calculated from the formula $h = 2\sigma/rdg$. The experiment demonstrates a water barometer. The bubbles appearing in the water when the vacuum was applied were very likely due to expansion of air bubbles present in the joints sealed with de Khotinsky cement.

WOODHOUSE reports a modification of the experiment in which a thread was run through the entire tube. In this case a continuous column was obtained unbroken by air bubbles. Presumably this column was supported by additional air pressure at the base since the author states that no tensile column was obtained yet shows a height of 14 meters in the figure (p. 188, **24**). The rate of flow (in a tube containing water and with a thread from top to bottom) when air pressure at the base (*i.e.*, vacuum at the top plus additional pressure at the base) is released and the tube allowed to empty is used as an argument for a combined tensile strength-sorption hypothesis. The curve obtained deserves attention. Extension across the abscissa indicates that with negative heights the velocity of descent continues to increase in contradiction to accepted laws of hydrostatics and free energy. It is difficult from the description of the experiment to explain this curve in any way. It is possible that the de Khotinsky cement extending into the lumen of the tube at certain points may have caused the results.

In modifying the tensile column theory, WOODHOUSE calls sorption into consideration, a factor whose importance in water transport in plants has been evaluated by SHULL (**15**). WOODHOUSE extends the sphere of influence

of the imbibitional or adsorptive forces of the wall material to the center of the smaller xylem vessels, suggesting a limiting distance of 5 microns for the action of this force. BODE (3) is quoted, " 'Dagegen war das Eindringen von Luft in Gefässe mit einem Durchmesser unter 10 μ nie zu beobachten.' " The heading of this portion of BODE's paper is "2. Die Wasserfäden in mechanisch verletzten Gefässen." In an earlier portion of his paper under the heading "1. Im intakten Gefäss" BODE states, "Ergebnis: Die Pflanze war vollkommen schlaff, und dennoch blieben sämtliche Wasserfäden intakt!!" This was the result after working on *Elatostemma sessile* which has vessels as large as 50 μ in diameter.

Aside from the fact that the hypothesis proposed by WOODHOUSE does not account for active conduction in vessels exceeding 10 μ in diameter, sorption, if acting to the distance and with the force postulated, is an *impediment rather than an aid* to water movement since imbibition forces sufficient to hold water against the pull of gravity would act as effectively along the tube against the pull of transpiration. In his hypothesis the tension of the water is suggested as the motive force while imbibition on the wall is one of support only. But, accordingly, to cause water movement the tensile force must overcome that of imbibition as well as that of gravity before motion can occur. If the imbibition forces are effective to the distance assumed by WOODHOUSE they should be sufficient to prevent the pushing of water through the 10 μ xylem vessels of a short piece of branch carefully cut at both ends to which a force of 1 atmosphere is applied. DIXON (6) obtained flow of water through wood of *Taxus baccata* using a head of water equal to the length of the branch. The diameters of the tracheids of this plant were not stated but presumably they were not much larger than 10 μ, while the diameter of the pores in the walls of the tracheids did not exceed 0.3 μ.

Experiments

To establish a complete water column supported by its adsorptive and cohesive forces the apparatus shown in figure 1 was set up. The tubes AB and CD were formed by melting with a gas-oxygen flame two Pyrex tubes 60 by 1.5 cm. and gradually drawing them out in a stair well into a tube of varying size, about 1.5 to 6 mm. inside diameter. The final length was 13.5 meters. After both tubes were drawn they were sealed together at the top so that a continuous glass siphon was formed. In order to insure sorption of the water on the glass, potassium bichromate in sulphuric acid was forced from D to A by means of a tire pump and valve at F. The flask used was a 250-cc. round-bottomed Pyrex flask. The rubber stoppers were wired in place to hold the pressure which was estimated by means of the manometer G. To obtain fairly rapid passage of the solution it was necessary to employ pressures of as much as five atmospheres. When the tube had been filled

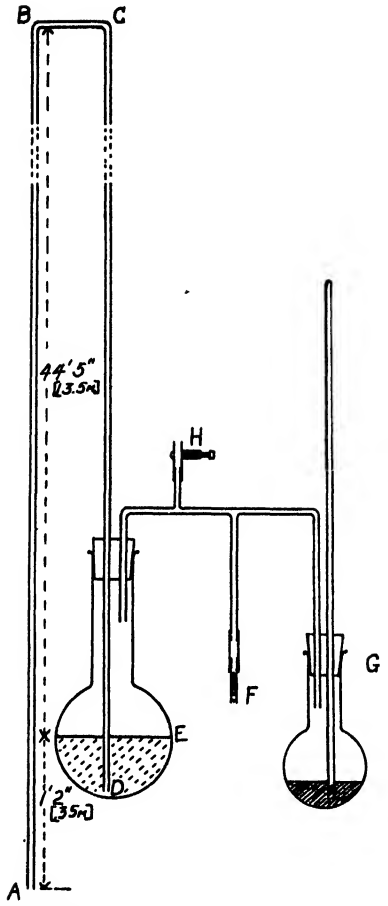


FIG. 1. Apparatus to demonstrate cohesion of water column.

with the cleaning solution alcohol was poured down the outside from the top and ignited when it reached the bottom. This heating of the solution in the tube insured thorough cleaning.

1. COHESION OF A COLUMN OF MOVING AIR-FREE WATER

The cleaning solution in the tube was followed by boiling water and this by boiled water which was allowed to cool in the tubes. The pressure in the flask was released by unscrewing the clamp H. The water column did not break and the 14-inch difference in level between A and E caused water to drip from A. After about 30 cc. of water left in the flask were emptied the ascending meniscus moved up the shorter arm DC with increasing speed as the difference in water level increased.

2. Cohesion of a column of moving air-containing water

The experiment was performed as before except that unboiled distilled water was substituted for the boiled water. This distilled water had been standing in a 5-gallon bottle open to the air. In this experiment the water column remained intact when pressure was released at the base and siphoned for six minutes, emptying 20 cc. of water at the base of the lower arm. The column then broke ten feet from the top of the tube DC, probably due to the conduction above 32 feet of some impurity in the distilled water. The flow before breaking was sufficient to show that the cohesive force of a moving solution of air in water is strong enough to support the water column to a height of 44 feet.

3. Force of water sorption in an agar gel

Tube AB was disconnected from CD at the top of the column and one end of a short piece of glass tubing 3 cm. in diameter was sealed on to the open top end. The junction of the small and the 3-cm. tubing was then narrowed to an area of about 3 sq. mm. The top of the 3-cm. tubing was left open to the atmosphere. Boiled water was forced up the tube after cleaning with cleaning solution and allowed to cool. The pressure at the base was maintained so that the water column was supported but did not change in level. Two per cent. agar solution was prepared and filtered while hot through a fritted glass filter. A piece of absorbent cotton was then dipped in the agar, taking care that no air bubbles were included in the interstices of the cotton which was then placed in the top of the water column and forced down into the constriction. The excess water in the top portion of the tube was replaced by agar and the cotton and agar allowed to set. The column remained intact when the pressure at the base was released.

It was originally hoped that evaporation from the surface of the agar would result in drawing water up the tube. To this end a waxed paper cylinder was tied over the open end of the tube containing the agar and connected to a beaker of sulphuric acid. The agar gradually decreased in volume and shrank away from the sides of the tube until at the end of 78 hours the shrinking allowed air to slip past the cotton-agar plug and the column broke. Only a part of the water leaving the agar gel was absorbed by the sulphuric acid, since 2 cc. of water were collected in a 10-cc. graduated cylinder placed at the bottom open end of the tube to measure the volume change. That this was not a volume change due to temperature difference was evidenced by the fact that the increase in water at the bottom was gradual during the time that the column was intact. The tension (less than $\frac{1}{2}$ atmosphere) at this height was sufficient to remove some of the water from the gel. The imbibition forces in the gel are insufficient to hold all the water against this pull. If the experiment had continued until equilibrium

had been established and no more water withdrawn, presumably evaporation from the surface of the agar would have resulted in pulling water up the tube.

Under such conditions of equilibrium the water bound in the gel by a force of about 0.5 atmosphere could be determined by drying the agar and weighing the residual water. Although at completion of the experiment equilibrium had not been reached, a considerable part of the water in the gel was withdrawn as is indicated by the figures (table I) for the 78-hour period.

TABLE I

Agar in the original gel	2	per cent.
Weight of agar, water, and cotton after 78 hours	6.40	gm.
" " " and cotton	0.46	gm.
" " cotton after boiling in water and drying	0.28	gm.
" " agar	0.18	gm.
" " water	5.94	gm.
Percentage agar after 78 hours of 0.5 atm. tension	2.94	per cent.
Percentage of water removed from agar during tension	32.7	per cent.

Of the water lost from the agar, 2 cc. were collected in the graduated cylinder at the bottom. The rest was absorbed in the sulphuric acid at the top of the column.

4. Cohesion of a stationary column of air-containing water

It was believed that difficulties in demonstrating the cohesive force of air-containing water were not so much due to dissolved air as to the presence of small particulate impurities with adsorbed air which might provide a nucleus for collection of dissolved air (**6**). The importance of such nuclei can be qualitatively observed in a dirty beaker in which tap water is heated as compared with a beaker cleaned in very hot cleaning solution. In the first case bubbles gradually collect on the walls of the beaker as the temperature rises, whereas in the second case few bubbles are to be observed. The influence of dirt on the boiling point was noted by Gay-Lussac [after Ursprung (**19**)]. Observations similar in principle indicating the importance of particles in allowing condensation of water vapor from the air are known from meteorological observations as well as from experiments in photographing electron paths.

Instead of boiling the water the results of experiment 3 suggested the possibility of filtering out all particulate impurities by means of the agar. A tube similar to that of experiment 3 was prepared except that it was drawn quickly from Pyrex tubing 3 cm. in diameter, forming a capillary 13.9 m. long, of diameter 0.6 mm. in the center to 1.4 mm. in diameter at a

distance 1 m. from the end. It was cleaned, filled with boiled, cooled water, and plugged with a cotton-agar plug. After the gel had set, the pressure at the base was released with no resultant break in the water column. Eight cc. of 0.25 per cent. acid fuchsin solution were poured on top of the agar. The top was closed to evaporation by tying over it a waxed paper bag. The layer of agar and cotton was about 2.5 cm. in thickness, tapering from a diameter of 3 cm. at the top to one of 1.5 mm. where the capillary joined the large tube. Below the constriction in the water-filled portion of the tube the diameter was 6 mm. In 8 days the acid fuchsin solution on top of the agar had been sucked through and an equivalent amount of water had collected in the flask at the bottom of the capillary. The red color of the dye could be observed extending from the agar down the capillary to a distance of 1 meter. Unless by some means the air in the dye solution was removed during the passage through the agar the water at the top of the column now contained dissolved air. It did not break.

To obtain a greater suction tension the container at the base of the capillary was gradually evacuated to determine at what tensile stress the column would break. When the manometer indicated 35.3 cm. Hg the sudden increase of water in the flask indicated that the column had broken. The break occurred at the junction of the agar-cotton gel with the water column. The barometric pressure was 75.3 cm. Hg. Calculation shows that the break occurred under a tension of 0.9 atmospheres. Thus a column of air-containing water is supported by its cohesive force to a height of at least 9 meters (19 meters when open to the atmosphere).

5. Effect of tension on viscosity of water in a capillary tube

In connection with the speeds of water movement found in plants (**8**), it is of interest to determine whether viscosity in a water column decreases with tension. Two capillary tubes were drawn out to a distance of about 35 feet in the same way as in experiment 4. These were sealed together at the top and at the base were sealed into flasks as indicated in figure 2. With the apparatus as shown it was possible to apply pressure or suction at will to either or both of the flasks at the bases of the capillary tubes and to count very accurately the flow of water by means of the drops from the capillary in the lower flask. After cleaning and filling with boiled distilled water the flasks and tubes were allowed to cool and then to siphon for two hours. By this time a temperature equilibrium with the surroundings had been reached.

Measurements were taken: (1) when the water in the flasks at the base was subjected to atmospheric pressure; (2) with an additional pressure of 40 cm. Hg applied to both of the flasks; (3) at atmospheric pressure as in

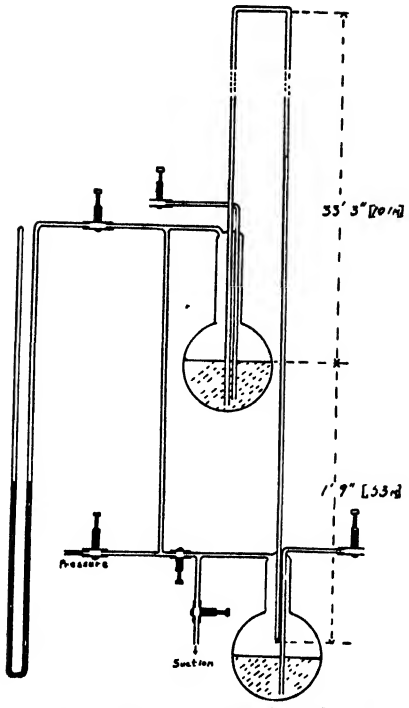


FIG. 2. Apparatus for measuring viscosity of water under tension.

1; and (4) with the flasks at the base both evacuated to a pressure of 27 cm. Hg. The results are given in table II.

Changes in viscosity with this tension change in the water are not sufficient to be detected with the method of measurement used. An experiment was started to determine the above rates with the use of a stopwatch but was discontinued when one of the flasks burst under the pressure used in filling the tube.

It is apparent that the method of measuring the siphoned water depends upon its surface tension as it drops from the lower capillary. In case the

TABLE II

MEASUREMENTS OF THE VISCOSITY OF WATER AT DIFFERENT TENSIONS

PRESSURE	TIME	NO. DROPS	RATE
76 cm. Hg	3 min. 55 sec.	20	11.75 drops/sec.
116 cm. Hg	3 min. 54 sec.	20	11.7 drops/sec.
76 cm. Hg	1 min. 57 sec.	10	11.7 drops/sec.
76 cm. Hg	1 min. 57 sec.	10	11.7 drops/sec.
27 cm. Hg	1 min. 56 sec.	10	11.6 drops/sec.

surface tension of the water changes in exactly the same way as the viscosity when subjected to varying tension or pressure the above experiment is invalidated. It would be necessary in such case to substitute some other means of measuring the water.

Discussion

To obtain consistent results in experiments in which the forces of adsorption and cohesion are concerned it is important to work with materials and surfaces that are clean and homogeneous. The variations in the experimental results obtained by various workers who have attempted to measure the cohesive forces of water are probably due to the variations in the degree to which they succeeded in cleaning the apparatus with which they worked.

In connection with impurities in the water it is important to distinguish between the state of different kinds of impurities. Gases, solids, and liquids dissolved in water are present in dispersed molecular or few molecular units. Any tendency of these impurities to lessen the cohesive force of the water is spread evenly throughout the mass of the water and not concentrated at a surface. (When concentration of the solute occurs at the surface as in soap solutions there is a very great effect on the surface tension and perhaps a corresponding effect on the cohesive force.) Some substances in solution may increase the cohesive force of the water.

The cell wall absorbs water with great force, insuring thorough wetting of the surface with which the water is in contact. That imbibing colloids such as the cell wall need not act to prevent movements of water through them when subjected to tension is indicated by the passage of the water through the agar gel. This may explain in part the way in which the transpiration stream travels from cell to cell in the water-conducting systems of some plants.

That a gel when subjected to a suction tension may shrink while still maintaining its organization and structure intact is indicated by the behavior of the agar gel. The relatively low suction tension force used in extracting water from the gel shows that the force of water sorption is low for a considerable proportion of the water held in the gel. Since the agar gel is a colloid the solid matter composing it may be assumed to be dispersed in units of about 5–200 mμ in diameter. It seems not unreasonable to assume that the diameter of the water-filled spaces in the solid phase does not exceed 200 mμ. The spaces are not visible microscopically. If the water spaces in the gel are of this size it must be concluded that the forces of water sorption of any magnitude are confined to a very short distance in the colloid since with a relatively low suction tension a considerable amount of this water can be removed. It seems probable that in the water-conducting cells of the plant great forces of water sorption act for very short dis-

tances and insure a wall which will act efficiently in excluding air bubbles and in providing an easily wetted surface, but they do not entirely impede the movement of water in the liquid phase within the colloid, much less in the lumen of conducting cells.

The results obtained in experiment 3 suggest that by using tensions of water a quantitative measure of the amounts of water held in certain colloids can be determined as well as the forces with which they are held. It may be that some modification of the scheme could be used to determine the amounts and forces concerned in the "binding" of water in non-living and perhaps in living systems.

The variations in trunk diameter of trees (**10, 7**) seem quite reasonable if we suppose that the tension of the water during periods of rapid transpiration draws a certain small amount of water out of the colloidal material of which the cell wall is composed. When the tension is released the imbibitional forces of the cell wall cause an absorption of water into the interstices of the colloid composing the wall and an increase in the size of the cell. It is of interest to speculate as to what effect the shrinkage of the cellulose in the wall might have on the ease with which water columns under heavy tension might be broken. It would seem that the shrinkage should decrease the ease with which air might penetrate into the vessels. Another effect of wall shrinkage may be to increase the rigidity of cells subjected to a tension so that leaf cells under normal tensions do not wrinkle and collapse but remain in approximately the same shape as when distended by turgor.

URSPRUNG and BLUM (**22**) have measured suction tensions in various parts of the plant and shown that the forces found are in accord with those needed in a cohesion theory of sap transport. To the writer this theory provides a coherent picture of the hydraulics of transpiration, a picture supported by many diverse experiments all indicating the simplicity and clarity of the central idea.

If we accept the physical forces operating at the surface of the leaf as the moving factors in supplying water to the plant, the constant wilting coefficient of a given soil for many kinds of plants finds a possible explanation. Evaporation from the mesophyll cells of the leaves draws ultimately upon the water in the soil. Since the walls of the evaporating cells of plants are composed of substances physically similar, celluloses, it is reasonable to conclude that they will have a similar maximum force which they can exert upon the water in the soil. Wilting occurs when the force with which water is held in the soil exceeds the force which the evaporating surface of the leaf can exert. Use of an instrument absorbing water through physical forces (**9**) has given a fairly constant value for the wilting points of various soils, a value which, if expressed in absolute amounts, is quite variable.

Summary

1. The results of certain experiments interpreted as throwing doubt on the tensile column theory of the ascent of sap can be explained as due to inadequate technique.

2. The tension-sorption hypothesis to explain ascent of sap during active transpiration is experimentally unsupported.

3. Reports of cohesive forces in air-free and air-containing water are substantiated.

4. A method is indicated by which bound water may be determined.

5. Within the limits of error of 1 per cent. no change in viscosity with a tension change of 1 atmosphere was found although the method of measuring the water may have hidden such changes.

6. A mechanism is suggested by which size changes without form changes may occur in both hard and soft parts of plants.

STANFORD UNIVERSITY

LITERATURE CITED

1. ASKENASY, E. Über das Saftsteigen. Verh. naturhist.-med. Ver. Heidelberg N.F. **5**: 325–345. 1895. Cited by WOODHOUSE.
2. BACHMANN, F. Studien über Dickenänderungen von Laubblätern. Jahrb. wiss. Bot. **61**: 372–429. 1922.
3. BODE, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefässpflanzen. Jahrb. wiss. Bot. **62**: 92–127. 1923.
4. BOEHM, J. Ursache des Saftsteigens. Ber. deutsch. bot. Ges. **7**: (46)–(56). 1889.
5. ————. Capillarität und Saftsteigen. Ber. deutsch. bot. Ges. **11**: 203–212. 1893.
6. DIXON, H. H. Transpiration and the ascent of sap in plants. London. 1914.
7. HAASIS, F. W. Seasonal shrinkage of Monterey pine and redwood trees. Plant Physiol. **7**: 285–295. 1932.
8. HARVEY, R. B. Tracing the transpiration stream with dyes. Amer. Jour. Bot. **17**: 657–661. 1930.
9. LIVINGSTON, B. E., and KOKETSU, R. The water-supplying power of the soil as related to the wilting of plants. Soil Science **9**: 469–485. 1920.
10. MACDOUGAL, D. T. Growth in trees. Carnegie Inst. of Washington. Pub. no. 307. 1921.
11. ————, OVERTON, J. B., and SMITH, G. M. The hydrostatic-pneumatic system of certain trees: movements of liquids and gases. Carnegie Inst. of Washington. Pub. no. 397. 1929.

12. MAXIMOV, N. A. The plant in relation to water. Translation by YAPP. London. 1919.
13. OTIS, CHARLES H. The ASKENASY demonstration. Plant Physiol. **5**: 419–423. 1930.
14. RENNER, O. Die Porenweite der Zellhäute in ihrer Beziehung zum Saftsteigen. Ber. deutsch. bot. Ges. **43**: 207–211. 1925.
15. SHULL, CHARLES A. Imbibition in relation to absorption and transportation of water in plants. Ecology **5**: 230–240. 1924.
16. SMITH, FANNY, DUSTMAN, R. B., and SHULL, C. A. Ascent of sap in plants. Bot. Gaz. **91**: 395–410. 1931.
17. THUT, H. F. Demonstration of the lifting power of evaporation. Ohio Jour. Sci. **28**: 292–298. 1928.
18. ———. Demonstrating the lifting power of transpiration. Amer. Jour. Bot. **19**: 358–364. 1932.
19. URSPRUNG, A. Zur Demonstration der Flüssigkeits-Kohäsion. Ber. deutsch. bot. Ges. **31**: 388–400. 1913.
20. ———. Über die Kohäsion des Wassers im Farnannulus. Ber. deutsch. bot. Ges. **33**: 153–162. 1915.
21. ———. Dritter Beitrag zur Demonstration der Flüssigkeitskohäsion. Ber. deutsch. bot. Ges. **34**: 475–488. 1916.
22. ———, and BLUM, G. Eine Methode zur Messung polarer Saugkraftdifferenzen. Jahrb. wiss. Bot. **65**: 1–27. 1925.
23. WALTER, HEINRICH. Der Wasserhaushalt der Pflanze in quantitativer Betrachtung. Freizing-München. 1925.
24. WOODHOUSE, E. D. Sap hydraulics. Plant Physiol. **8**: 177–202. 1933.

FREEZING PHENOMENA IN CRESOAP EMULSIONS OF PETROLEUM OILS[1]

PAUL A. YOUNG

(WITH PLATE II AND TWO FIGURES)

Introduction

Studies of freezing emulsions were included in the experiments conducted during 1928 to 1933, to determine whether freezing of oil sprays on apple buds increased the injurious effects of the oils. The protoplasm in the buds is a hydrosol resembling oil-in-water emulsions, so these liquids may behave similarly in freezing. Hence the phenomena of freezing in oil-in-water emulsions were studied to try to reveal information helpful in elucidating the effects of freezing in protoplasm.

Materials and methods

Emulsions of oils 4, 16, 24, and the other oils described in table I, were frozen at $-3°$ to $-30°$ C. in many experiments. Some samples of oils 16 and 24 were saturated with the oil red O stain before emulsification. The emulsions were studied while they were frozen, and also while they were freezing and melting, to observe how they differed from unfrozen emulsions.

Distilled water, and 0.25 per cent. of amorphous carbon suspended in distilled water, were frozen separately for comparison with frozen emulsions. Gas was removed from eight emulsions and from boiled distilled water by evacuating them with a Cenco Rotovac pump before these liquids were frozen. Many emulsions were frozen and melted two or three successive times to determine whether this increased the amounts of oils liberated.

Oils 4, 16, 24, and 36 were made miscible by dissolving 5.5 per cent. of potassium-fish-oil soap and 4.5 per cent. of cresols in them in making the stocks of cresoap-miscible oils (**1**). A high-speed electric stirrer and warm tap water were used in emulsifying the miscible oils and in diluting the emulsions.

Emulsions were frozen in 170 test tubes having dimensions of 100–300 by 15–25 mm., and on 50 microscope slides with and without cover glasses. Thick layers of emulsions were made on some slides by supporting the cover glasses with fragments of broken cover glasses. Melting of emulsions was studied by holding hot needles near them.

[1] Paper no. 36 of the Journal Series of the Montana Agricultural Experiment Station. Contribution from the Department of Botany and Bacteriology.

TABLE I

PETROLEUM OILS USED IN THE STUDIES OF FREEZING EMULSIONS

OIL NUMBER	NAME OF OIL*	PERCENTAGE SULPHONATABLE	VISCOSITY†	PERCENTAGE OIL IN EMULSIONS
		%	*sec.*	%
4	Standard (Cal.) Mineral Seal 4, 13607R	10	50	4
16	Shell 3	6	55	1, 2, 4, 8
24	Sonneborn Amalie 10680P	0	67	2, 10
29	Standard (Ind.) Dendrol Miscible...	30	83	4, 8
30	Standard (Ind.) Verdol Emulsion...	4	83	5.5
36	Shell E515	14	75	4, 8

* The writer wishes to thank the Standard Oil Co. (Cal.), Standard Oil Co. (Ind.), Shell Oil Co., and L. Sonneborn Sons, Inc. (N. Y.), for supplying the oils and the information about the viscosities and sulphonatable residues of the oils.

† Viscosity in seconds determined with the Saybolt Universal viscosimeter.

Experimental results

EMULSIONS FROZEN ON MICROSCOPE SLIDES

The emulsions frozen on microscope slides had the macroscopic appearance of feathery frost on window glass (fig. 1).

Magnifications of 50 to 600 times revealed the following sequence of phenomena in the cresoap emulsions of 2 and 4 per cent. oils 4, 16, and 24 while they were frozen, and while they were freezing and melting:

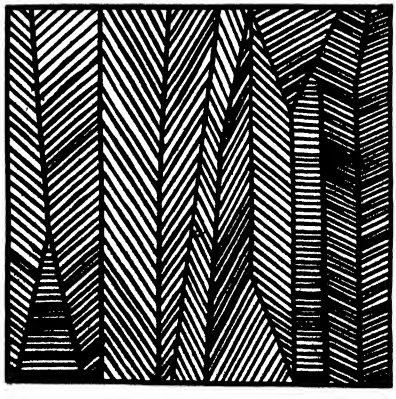


FIG. 1. Diagrammatic drawing of a cresoap emulsion of 2 per cent. oil frozen on a microscope slide. Black lines between transparent ice crystals represent main and branch channels of oil, gas, and aqueous solution of cresols and soaps. ×10.

a) Long needles of ice rapidly invaded fields of emulsions, crowding the oil globules into narrowing channels between the ice crystals, and pressing many oil globules together until they coalesced to form elongate masses of oil (fig. 2 *A*). Oil globules and masses occurred in straight rows as long as 1.7 mm. Gas was evolved while ice crystals formed in freezing emulsions (fig. 2 *D*).

b) Spaces containing gas or aqueous solutions appeared as blue lines nearly 0.2 μ wide between adjacent ice crystals (fig. 2 *B*). Light from these lines was refracted by the microscope lens, making the lines look blue. Horizontal lamellae of ice apparently formed first on the bottoms of the emulsion layers on slides, probably because the slides were laid on ice or stone presumably colder than the surrounding air. Impurities were forced into the liquid above these original crystals so that the crystals touched and formed the blue lines.

c) New horizontal lamellae of ice formed on this bottom layer until all available water was crystallized. Many of these superimposed crystals were separated by wide channels of gas, and by channels of aqueous solution of gas, cresols, and soaps surrounding masses of oil (fig. 2 *C, D, E*). These channels were compressed between the ice crystals, and lay above many of the blue lines.

d) As frozen emulsions melted, widening channels of clear water replaced the blue lines. The channels of oil, gas, and aqueous solution widened as ice crystals melted, and the elongate bodies of oil and gas suddenly became circular in outlines. Many gas bubbles 1 to 20 μ in diameter dissolved and disappeared in the warming water.

e) Oil globules moved rapidly and dispersed in the widening channels of water between melting crystals of ice. Many of the large masses of oils partly or entirely re-emulsified in the water liberated from the ice. Freezing and melting apparently increased slightly the predominating sizes of the oil globules on many slides.

The channels of oil, aqueous solution, and gas between ice crystals were straighter and less numerous in the frozen emulsions of 2 per cent. oil than in those of 4 per cent. oil. Many masses of oil were not inclosed in the channels between ice crystals (fig. 2 *D*). Some of these masses appeared to lie between the ice and the cover glass. Ice frozen from the evacuated emulsions had fewer gas channels and bubbles than ice frozen from the unevacuated emulsions.

Aqueous solution, oil, and gas in the channels between the ice crystals had similar appearances, but dark-field illumination left only the oil prominently visible. Oils also were made distinguishable when freezing precipitated the oil red O in them, and when melting of ice crystals released irregular masses of oils.

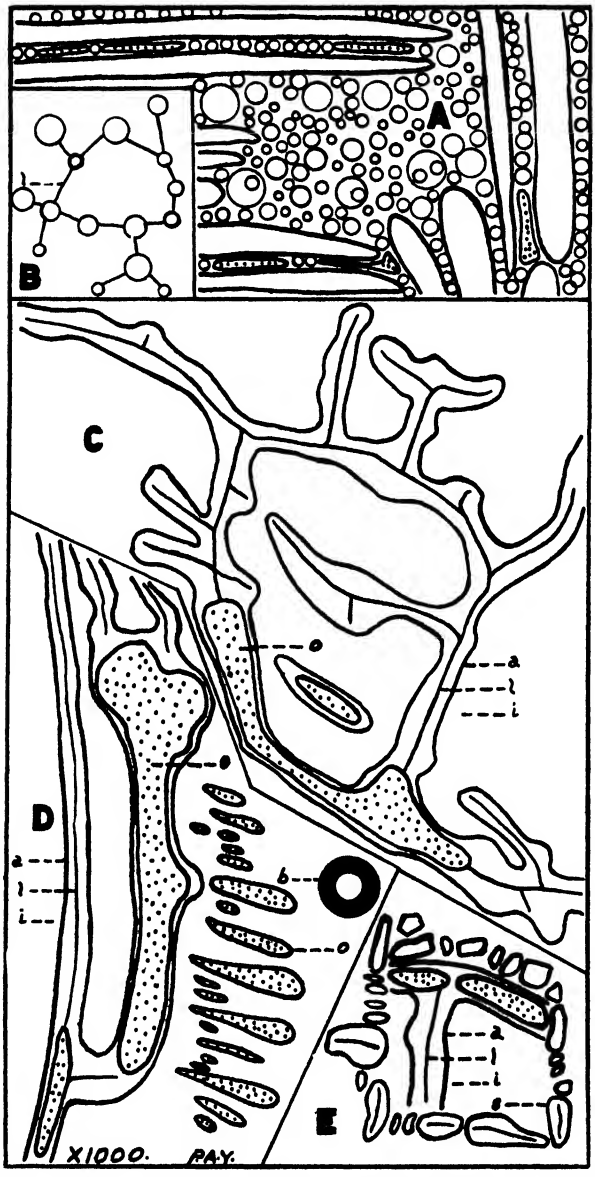


FIG. 2. Diagrammatic drawing showing common arrangements of ice, oil, gas, and aqueous solution of gas, soaps, and cresols in cresoap emulsions of 2 and 4 per cent. oils frozen on microscope slides. Stippled areas represent irregular masses of oil deformed by ice crystals. In parts *B* to *E*, the lines (*l*) represent the blue lines of gas and liquid between nearly touching ice crystals.

A. Needle-shaped crystals of ice invading a field of oil globules (circles). Six irregular masses of oil are compressed between ice needles.

Similar freezing phenomena developed in the emulsions of oils 4, 16, and 24, and oils 16 and 24 saturated with oil red O. These phenomena also were similar in thick and thin layers of emulsions on slides with and without cover-glasses.

Water and carbon suspension frozen on microscope slides

Unlike the ice in frozen emulsions, the ice that formed in boiled evacuated distilled water showed only a few wide channels of gas, and probably a little liquid water saturated with gas. Most of the blue lines separating crystals were alone, but wide channels of gas lay above a few of them. Gas bubbles rose from some of the blue lines (spaces) between the ice crystals when this ice melted and the water became warm. Frozen tap water was similar, but had many more of the wide channels of gas above the blue lines.

The frozen suspension of amorphous carbon exhibited the carbon particles aggregated with gas in the channels; the phenomena otherwise resembled those in frozen tap water. Melting the ice on two of the slides left polygons of carbon particles apparently adhering to the slides.

Emulsions frozen in test tubes

The emulsions freezing in test tubes displayed the following sequence of phenomena:

a) Layers of transparent ice 1 to 7 mm. thick formed near the glass, concentrating the oil globules, gas bubbles, and aqueous solution of gas, cresols, and soaps near the centers of the tubes (pl. II *F*). Parts of the outer transparent ice commonly contained long, parallel, or radial pockets of gas.

b) Needles (pl. II *A*), lamellae (pl. II *D*, *E*), and polyhedrons (pl. II *A*, *C*) of ice formed in the emulsions, concentrating the oil globules.

B. Spherical globules of oil (circles) above the corners of ice-polyhedrons.

C, D. Frozen cresoap emulsion of 4 per cent. oil 4 showing ramifying channels (*a*) of gas and unfrozen aqueous solution of cresols and soaps, lying over blue lines (*l*) between ice crystals (*i*). Higher lamellae of ice inclose and determine the forms of the channels. Irregular masses of oil (*o*) are inside and outside the channels. The thick-margined circle (*b*) represents a large uncompressed sphere of gas.

E. Frozen cresoap emulsion of 4 per cent. oil 16 saturated with oil red O showing masses of unfrozen aqueous solution of cresols and soaps (*s*) arranged as discontinuous channels.

C, D, E. Emulsions frozen at $-6°$ C. The channels (*a*) had dimensions of 10–645 by 5–30 μ. The main channels were 2.5–225 μ apart and the branch channels were 8 to 75 μ apart. The irregular oil masses (*o*) had dimensions of 35–120 by 7–21 μ, and the spherical oil globules were 0.5–20 μ (mostly 1–8 μ) in diameter. Before the cresoap emulsions were frozen, oil globules 0.1–8 μ in diameter predominated in them.

gas bubbles, and aqueous solution of gas, cresols, and soaps between these compound ice crystals (4). The lamellae of ice resembled those frozen in dairy cream and milk. Lamellae, needles, and polyhedrons of ice were not conspicuous in part of the emulsions. Ice columns looked milky and opaque during this stage because milky emulsions remained between the ice crystals.

c) Further freezing enlarged the ice crystals by removing water from the channels until the milky-ice columns became translucent and non-milky, apparently because the lamellae and polyhedrons of ice held masses of oils, gas, and concentrated aqueous solution of gas, soaps, and cresols in such narrow layers and channels between them (pl. II *C, D, E*). During this stage the ice columns expanded and pressed against the curved bottoms of the test tubes. This forced the ice columns upward, and usually prevented breaking of the glass tubes. However, rapid freezing of emulsions at – 20° to – 30° C. broke many test tubes.

d) One to four horizontal cracks developed in most of the expanding columns of freezing emulsions (pl. II *A, B, E, F*). The formation and shapes of these cracks were not altered by making constrictions or bends in the test tubes, or by inclining the tubes at 45° angles instead of holding them in the usual vertical positions. The cracks were milky at first, and developed in non-milky columns of ice. Then ice needles and lamellae entered the cracks and they also became non-milky ice. Red-stained oil indicated that the cracks contained less oil than the other parts of the ice columns. Many ice columns were slipped from their test tubes and were broken easily only at the cracks.

The formation of milky emulsion was the first macroscopic evidence of melting of frozen emulsions (pl. II *D*).

Microscopical observations on the melting of the frozen emulsions showed that the oil globules 0.1 to 20 μ in diameter usually became redispersed in water liberated from the ice. Also many of the larger masses of oils partly re-emulsified in the water. Convection currents in water melting from ice probably facilitated emulsification.

Freezing and melting, especially when repeated, liberated 60 to 90 per cent. of the oils in the emulsions of 1 and 2 per cent. oil; 5 to 10 (sometimes 50) per cent. of the oils in the emulsions of 4 per cent. oil; but apparently liberated no oils from the emulsions of 8 per cent. oil. The emulsions were not agitated artificially while freezing and melting.

Frozen columns of emulsions of 4 per cent. oils 4 and 16 were allowed to remain undisturbed in their test tubes while melting and during a week after melting. The liquids in many of these tubes showed prominent layers bearing different concentrations of oils in the following order from top to bottom: (a) undiluted oil, (b) cream, (c) concentrated emulsion, (d) dilute milky emulsion, and (e) transparent emulsion.

No differences were seen in the freezing phenomena in the emulsions of oils 4, 16, 24, 36, 16 saturated with oil red O, and 24 saturated with oil red O, except that the red-stained oils made pink milky emulsions and red ice.

Freezing an emulsion of 67 per cent. oil 16, emulsified by casein and ammonia, produced many large lamellae of ice like those shown in pl. II *E*. This ammonia-casein-oil emulsion apparently was unaltered by the freezing and melting.

The frozen suspension of 0.25 per cent. amorphous carbon in test tubes showed the carbon particles and gas bubbles to be concentrated in long narrow pockets in the centers of the ice columns. Irregularly horizontal cracks developed in the ice columns. Melting left nearly all of the carbon particles in the bottoms of the test tubes.

Emulsions frozen on apple buds

Emulsions of 4, 6, 8, and 16 per cent. oils froze on apple buds in experiments conducted during 1928 to 1931. There was no evidence that the freezing increased the injurious effects of the oils. The oils emulsified by calcium caseinate usually are liberated quickly without freezing, and most of 4, 6, and 8 per cent. oils emulsified by cresoap re-emulsify in melting, so that freezing is not expected to make oil emulsions more injurious.

Discussion

The following concepts help to explain the freezing phenomena in cresoap emulsions of petroleum oils, and the related phenomena in freezing protoplasm.

In freezing, liquid water excludes other molecules and ions, and forms pure ice when the attractive forces of crystallization between water molecules exceed the forces which prevent freezing. These attractive forces predominate and crystallize pure uncompressed water when enough heat is lost to decrease sufficiently the thermal motions of molecules, and when the latent heat of fusion is liberated. While the thermal motions in solutions decrease below 0° C., the attractive forces between water molecules evidently increase until they overcome the attractions between water and other molecules and ions, and overcome pressures which hinder crystal expansion. Undercooling, pressure, and the existing concentration of non-aqueous molecules, ions, and colloidal particles adsorbing water determine the temperature at which water starts to crystallize below 0° C. This describes phenomena that occur when freezing is relatively slow.

In this way freezing emulsions drove gas from solution and concentrated it with oil and aqueous solution of cresols and soaps in channels and

cavities between compound crystals of ice. Most of the emulsions were undercooled before freezing. Also, pressures strong enough to move and break the ice columns, and break some of the test tubes, developed in the freezing emulsions. Large masses of oil, formed by aggregation of small oil globules, were common in channels in the frozen emulsions. Many small oil globules remained suspended in concentrated aqueous solution of gas, soaps, and cresols between ice crystals. Much oil probably dissolved in such solutions (**3**).

Strong interfacial membranes occur on oil globules containing cresoap. When such oil globules are pressed together, polar radicals on the oriented interfacial soaps probably hold films of water between the oil globules many of which only apparently instead of really coalesce. Compacted, distorted oil globules abound in emulsions of more than 75 per cent. oil (**3**). This helps to explain why melting cresoap emulsions displayed redispersion of small oil globules and partial or nearly complete re-emulsification of large oil masses. Cresoap emulsions of 4, 8, and 10 per cent. oil are more stable than those of 1 and 2 per cent. oil, and this stability characterizes these emulsions during freezing and melting as well as during exposure to constant temperatures.

The cresols and soaps are soluble in water, so presumably they diffuse from globules of cresoap-miscible oils into the water in the emulsions. Minute bubbles of gas also are dispersed in emulsions.

SWINGLE and SNAPP (**2**) determined freezing points of $-0.1°$ to $-1.4°$ C. for different emulsions of $\frac{2}{3}$ oil in water. Apparently the ordinary emulsions cannot be undercooled below $-8.3°$ C. before freezing begins. The freezing points were governed by the emulsifiers.

The observations and interpretations of freezing phenomena in oil emulsions add support to the following concepts in literature about the effects of freezing on protoplasm: (a) Freezing may reduce the water phase below the limit of tolerance in protoplasm unprepared for such dehydration. Naturally increasing the concentrations of solutes and hydrophilic colloids in protoplasm retards and decreases the release of water in freezing and thus lowers freezing points of protoplasm. (b) Ice crystals rupture and crush cell structures.

Further information about freezing will be valuable in studying refrigeration and winter hardiness of plants.

Summary

1. Microscopic freezing phenomena in cresoap emulsions of 2 and 4 per cent. oils on microscope slides were: (a) Needles and lamellae of ice invaded fields of oil globules, arranged them in rows, and caused many of them to coalesce. (b) Compound ice crystals enlarged until adjacent ones were

separated only by narrow spaces which appeared as blue lines. (c) Above these blue lines, other layers of ice inclosed irregular masses of oils, gas, and aqueous solution of gas, cresols, and soaps in irregular channels.

2. As thermal motions of molecules decrease below 0° C., the attractive forces causing crystallization increase until they exclude impurities and aggregate the hydrogen and oxygen of water into crystals of pure ice. This explains theoretically why the dissolved oxygen, nitrogen, cresols, and soaps were excluded from crystallizing water. Removal of the water concentrated these impurities, and crystal expansion inclosed them between the ice crystals.

3. Emulsions frozen on slides had the macroscopic appearance of feathery frost on glass windows.

4. Macroscopic freezing phenomena in cresoap emulsions of 2, 4, and 8 per cent. oil in test tubes were: (a) Clear ice with or without gas bubbles formed near the glass and concentrated the emulsions near the centers of the tubes. (b) Needles, lamellae, and polyhedrons of compound ice crystals formed, inclosing the oils, gas bubbles, and aqueous solutions of emulsifiers in the interstices. (c) The freezing columns of emulsions changed from milky to non-milky appearance, expanded, and formed usually horizontal milky cracks that later froze and also became non-milky.

5. Freezing and melting liberated 60 to 90 per cent. of the oils from emulsions of 1 and 2 per cent. oil; 5 to 10 (sometimes 50) per cent. of the oils in emulsions of 4 per cent. oil; but apparently liberated no oils from emulsions of 8 per cent. oil. Oil globules and large parts of the irregular masses of oil re-emulsified when the ice melted.

6. Freezing of emulsions on apple buds apparently did not increase oil injuries.

The writer wishes to thank Dr. W. M. MARTIN and Dr. R. A. MELAVEN of the Chemistry Department, and Professors A. J. M. JOHNSON and J. A. KIEFER of the Physics Department of Montana State College for their valuable suggestions.

MONTANA AGRICULTURAL EXPERIMENT STATION
BOZEMAN, MONTANA

LITERATURE CITED

1. MELANDER, A. L., SPULER, A., and GREEN, E. L. Oil sprays: Their preparation and use for insect control. Washington Agr. Exp. Sta. Bull. 184. 1924.
2. SWINGLE, H. S., and SNAPP, O. I. Petroleum oils and oil emulsions as insecticides, and their use against the San José scale on peach trees in the South. U. S. Dept. Agr. Tech. Bull. 253. 1931.

3. WOODMAN, R. M. The physics of spray liquids. IV. The creaming capacity of emulsions-paraffin solutions. Jour. Pom. & Hort. Sci. **4**: 184–195. 1924.

4. YOUNG, P. A. Lubricating-oil sprays and their effect on potatoes. Phytopath. **22**: 31. 1932.

EXPLANATION OF PLATE II

A. Needles and polyhedrons of ice with milky emulsion between them shown in a freezing cresoap emulsion of 4 per cent. oil 16 saturated with oil red O. Horizontal crack formed near the base of the ice column. ×2.

B. Horizontal crack formed in ice frozen from the emulsion described in *A*. ×2.

C. Polyhedrons and lamellae of ice with concentrated layers of a cresoap emulsion of 8 per cent. oil 16 between them. ×2.

D. Oblique lamellae of ice with concentrated emulsion layers of 8 per cent. oil 29 between them. The emulsion began to melt during photographing and released some milky emulsion. ×1.

E. Vertical lamellae of ice with layers of a cresoap emulsion of 8 per cent. oil 16 between them. Horizontal cracks are shown. ×2.

F. Translucent, non-milky ice surrounding a central column of milky ice, with one horizontal crack in a frozen emulsion of 5.5 per cent. oil 30. Oil globules 2 to 12 μ in diameter predominated in the emulsion before freezing. The melting occurred in the outer layer of non-oily ice, so masses of water with circular and elliptical outlines appeared near the glass; this contrasts with the milky emulsion shown in *D*. ×1.

YOUNG: CRESOAP EMULSIONS

PRODUCTION OF VITAMINS BY A PURE CULTURE OF *CHLOROCOCCUM* GROWN IN DARKNESS ON A SYNTHETIC MEDIUM

M. F. GUNDERSON AND C. E. SKINNER

(WITH TWO FIGURES)

Introduction

Although considerable information has accumulated with regard to the occurrence of vitamins in plants and animals, little pure culture work has been done for the purpose of studying the physiology of their production by plants.

The data reported here are concerned with a species of *Chlorococcum*,[1] a green alga, isolated from soil. The organism can be grown on a purely inorganic substrate with natural or artificial light as the source of energy, or it can be cultivated, as in these experiments, in complete darkness with a carbohydrate as the source of energy. By using algal cells grown in this manner, it is possible to separate the factor of light from that of chlorophyll formation which takes place under such conditions even in the absence of light (**2, 17**).

Recent investigations (**4, 7, 12**) on the synthesis of vitamin A in land plants show that in the early stages of germination in the dark no vitamin is formed, but that after 10 to 16 days it is present in etiolated shoots. Light was found to accelerate the formation of the vitamin.

Vitamin production by unicellular algae (diatoms) has hitherto been observed (**1, 9**), but light was not excluded and the components of the nutrient medium were not limited to but one source of organic material, dextrose, for vitamin synthesis. An earlier paper (**18**) has demonstrated the production of vitamin A or its provitamin by a bacterium in darkness on a glucose-peptone substrate.[2] Even at the present time (**11**) vitamin A synthesis is associated by many people with the presence of light. In this paper the data do separate the factor of light from vitamin formation.

The well recognized ability of yeast to synthesize vitamin B (**13**) and G (**19**) has demonstrated that neither light nor pigment is essential in the

[1] This organism (no. 7 in our collection) was identified by Dr. FELIX MAINX of the German University at Prague, Czechoslovakia. Pure cultures have been placed in the museum of Prof. E. G. PRINGSHEIM of the same institution, from whom transfers may be obtained for a nominal sum.

[2] This has recently been confirmed by others. Jour. Biol. Chem. **103**: 339–351. 1933; Jour. Bact. **28**: 31–40. 1934.

formation of these factors. Vitamin B (old terminology) has been found in all species of bacteria and fungi tested (**20**). The presence of vitamin B has been investigated (**7**) in seeds, etiolated seedlings, and green plants, with the use of different light sources, with the resultant conclusion that no increase was obtained during germination and that the formation of vitamin B came at a later period during the development of the plant.

It early became known (**5**) that germinated seeds were strongly antiscorbutic. Light was shown to play no part in the formation of vitamin C (**3**). Later work indicated that oxygen was necessary for the formation of vitamin C (**8**), and that its concentration was increased by exposure to light (**7**).

Experimental work (18, 16, 6)

Pure cultures of the alga were grown upon a medium of the following composition (BEIJERINCK'S):

NH_4NO_3	0.5 gm.	$Fe_2(SO4)_3$	1 drop 1/10 saturated water solution
K_2HPO_4	0.2 gm.	Glucose (Difco)	10 gm.
$MgSO_4.7H_2O$	0.2 gm.	Agar-agar (Difco)[a]	15 gm.
$CaCl_2$	0.1 gm.	Distilled water	1000 cc.

Approximately 100 cc. of this medium were placed in 32-oz. medicine bottles and sterilized at 18 lb. pressure for 20 minutes. The bottles were then laid flat and the agar permitted to solidify, thus presenting as large a surface as possible. This medium was inoculated and the resulting algal growth harvested as already described (**6**). Methods for drying other than the one described could be devised to reduce opportunities for oxidation.

The flasks were incubated at room temperature in cupboards protected from light by piling the flasks in tiers on trays, the cotton-stoppered ends facing the tight-fitting wooden doors of the cupboards. There was no growth on inoculated control tubes of the same medium without sugar or agar which were placed in this same environment. This fact indicates that the darkness was complete and that the algae grown for the experiments led a completely saprophytic existence. Owing to the lengthy incubation period of five or six weeks, some cultures became contaminated and were discarded. Contaminants in the culture flasks were easy to detect, since bacteria or molds spread very rapidly over the surface during the protracted incubation period.

The algae were harvested using the method already referred to, and the

[a] The agar was further purified by washing in a continuous extractor for 48 hours with alcohol, then 24 hours with ether.

algal paste obtained was dried in petri dishes in a current of warm air. The algal mass was ground to pass through a 1-mm. sieve, and kept in a well stoppered bottle in a mechanical refrigerator. One hundred and fifty grams of dried algae may be obtained from five hundred culture flasks.

Extreme care was exercised in the growing, harvesting, and drying of the material to prevent bacterial growth; in fact, the only time bacterial growth could have occurred was while the material was being dried. This drying was complete enough for grinding within 36 hours, and the moisture content of the mass was lowered below that required for bacterial growth in much less time. By plating the material, it was found that the bacterial count was much less than that of the rations fed or of the drinking water remaining in the usual glass drinking fountains after the rats had contaminated them with food particles.

The rats used for testing the vitamin A content of the algae were the progeny of a colony which are maintained on a diet designed to minimize the amount of this vitamin the rat is able to store, but does supply a sufficient amount for growth, reproduction, and lactation. The general laboratory technique and methods for the preparation of food materials used in this investigation have been described (**14**) by others.

The compositions of the rations used in testing for vitamins A, B, and G are given in table I.

TABLE I

COMPOSITION OF RATIONS

	A-FREE*	B-FREE†	G-FREE‡
Casein	18.0	18.0	18.0
Salts§	4.5	4.5	4.5
Agar-agar	2.0	2.0	2.0
Butter fat	0.0	10.0	10.0
Crisco	10.0	5.0	5.0
Tapioca-dextrin	65.5	62.5	62.5
	100.0	102.0	102.0

* Vitamins B and G were supplied by 0.5 gm. dried yeast fed separately from the ration.

† Vitamin G was supplied by 0.5 gm. yeast which had been autoclaved 3½ hours at 15 lb. pressure and given separately from the ration.

‡ Vitamin B was suppliel in the form of tiki-tiki extract made according to the method of Williams, dried on dextrin, and fed separately.

§ McCOLLUM salt mixture, no. 185, to which had been added 0.8 per cent. $CaCO_3$ in each 100 gm. of ration.

Vitamin D was supplied in the B- and G-free rations by exposing the ration for 15 minutes, at distance of 2 ft., and in a thin layer, to a mercury vapor quartz lamp operating at 110 volts and 5 amperes.

Vitamin A

In testing the algae for vitamin A content, fourteen 28-day old rats were maintained on the vitamin A-free ration for seven weeks, when de-

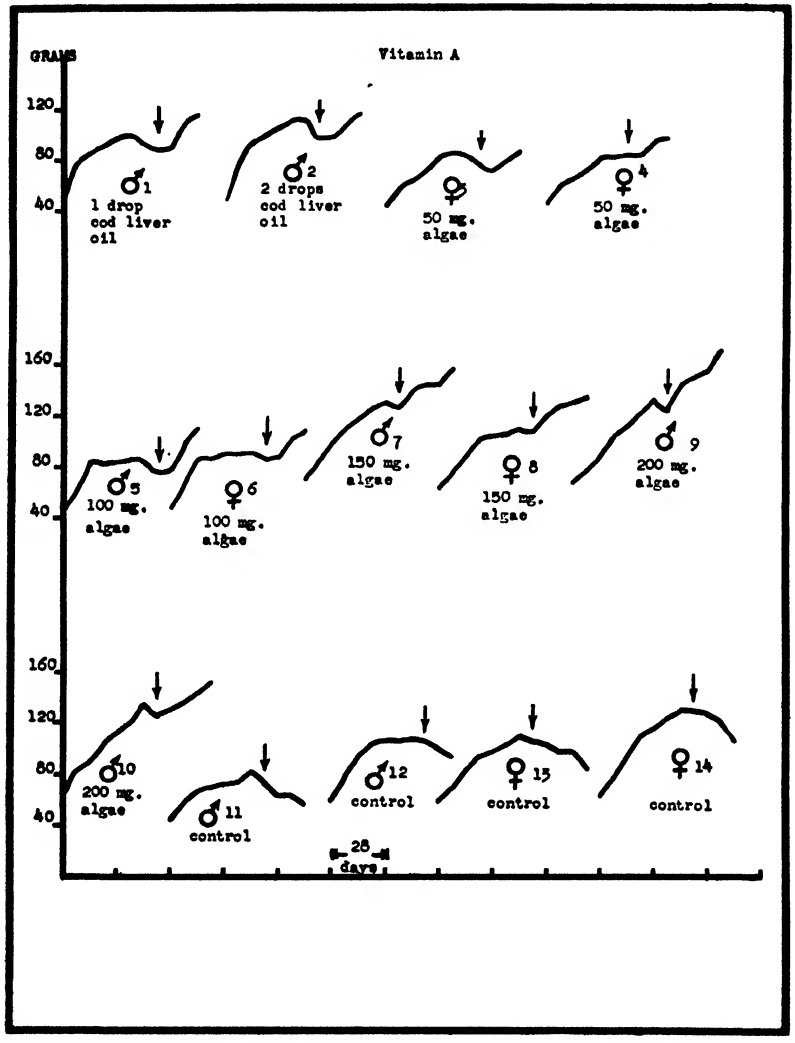


Fig. 1. Curative experiment, vitamin A: Arrows indicate point at which the feeding of the curative was started, except in the case of the controls, where they indicate the points at which the same number of weeks of experiment were reached.

cline in weight and xerophthalmia gave assurance of vitamin A deficiency. The rats were then divided into groups of two each, and different weights of the curative portions given as indicated in figure 1.

The growth curves of the rats receiving the algae as a curative show that 50 mg. of algae are inferior to the amounts of cod-liver oil fed in causing resumption of growth. With increasingly larger doses of algae there were correspondingly greater increases in the rate of growth. Three of the four rats which served as negative controls died from effects of the deficiency before the end of the experiment. The rats in the experiment were irradiated daily for two to five minutes to supply the vitamin D requirement.

Records of the food intake for the individual rats beginning at the time the curative portions were given show that the rats receiving 50 mg. and 100 mg. portions of algae ate less than an average of 40 mg. of ration per week, and those which received 150 mg. and 200 mg. of algae ate an average of 40 gm. per week.

Vitamins B and G

Seven rats, 28 days old, were maintained on the diet described, lacking both vitamins B and G. At the end of the eighth week the rats were placed in separately caged groups, and the supplement fed in addition to their ration. The yeast was fed in the form of pills while the algae was given in its powdered form in a carefully weighed portion for each rat. At the beginning of the experimental period the rats appeared to be suffering from inanition and malnutrition. From the manner in which these rats responded, both in weight and increased appetite, it could be concluded that the algae contained vitamins B and G.

Each of the vitamins was tested for separately, as indicated in the following experiments.

Vitamin B.—Three rats, 28 days of age, were maintained on a vitamin B-free basal diet throughout the experiment, as already explained. The rats were allowed to continue on this diet until avitaminosis was apparent. At the end of the fifth week, rats 1 and 3 were considered to be in a condition to warrant the addition of 0.5 gm. of algae to their diet daily. In the case of rat 2, it was not until the end of the twelfth week that the algae was fed.

Vitamin G.—Three rats, 28 days old, were maintained on the vitamin G-free ration. When the effect of the vitamin G deficiency was obvious, 0.5 gm. of algae was fed to each rat daily.

From the results obtained (fig. 2) it was concluded that vitamin B and G were present.

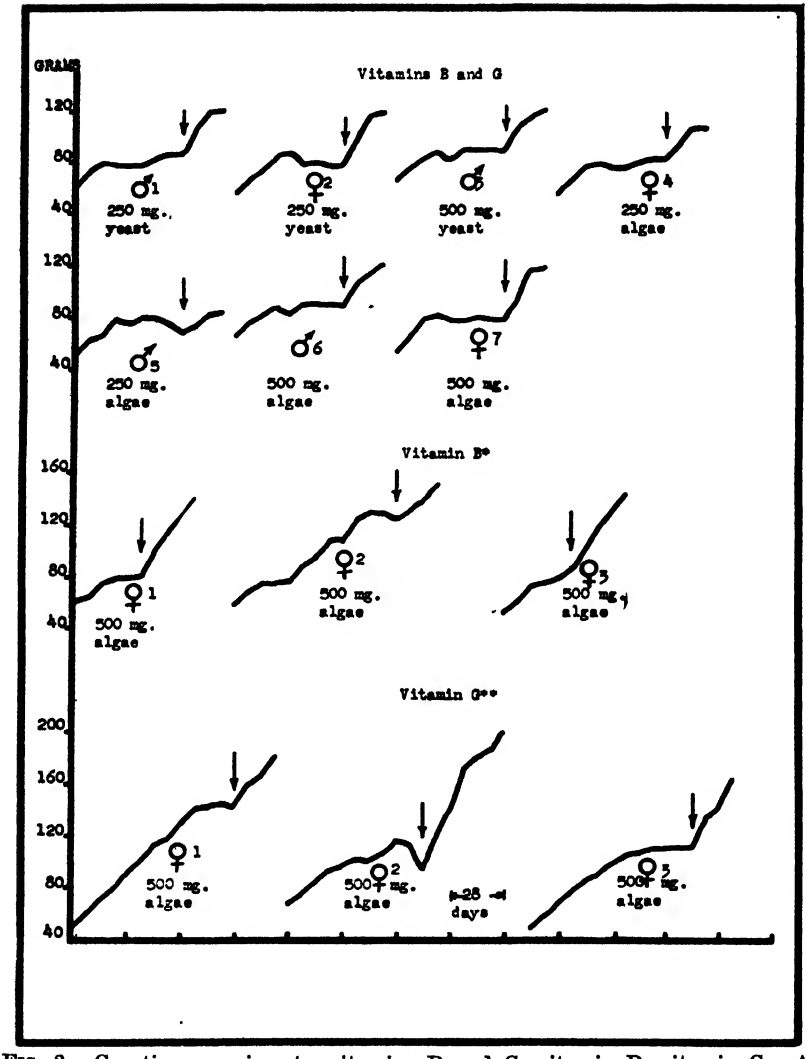


FIG. 2. Curative experiments, vitamins B and G, vitamin B, vitamin G: Arrows indicate point at which feeding of curative was begun.

* 0.5 gm. of autoclaved yeast was fed daily to supply vitamin G.

** 0.5 gm. of tiki-tiki (dried) was fed daily to supply vitamin B.

VITAMIN C

Six guinea pigs were fed a scurvy-producing ration which consisted of:

Ground yellow corn	3 parts
Ground rolled oats	3 parts
Oil meal	1 part
$CaCO_3$	1 per cent. of the ration
NaCl	1 per cent. of the ration

Prairie hay was also given *ad lib.* The algae which were used as the antiscorbutic had not been dried. The algal growth was removed from the agar by adding a small amount of water to the culture flask and shaking the flask after loosening the growth from the agar with a scraper. The resultant suspensions were centrifuged. The supernatant fluid from the centrifuge cups was used repeatedly, during the few hours required to make a harvest, to wash the algae from other bottles. Thus as little liquid as possible was used in making the harvest. The final liquid was of a syrupy consistency and had a moisture content of 90.7 per cent. The product was kept in an efficient refrigerator.

Two guinea pigs were fed 3 gm. each of the algal suspension daily, equivalent to 0.29 gm. of dried alga; two were given 5 cc. each of tomato juice; two guinea pigs were not given any supplement and served as negative controls. Other guinea pigs were kept on a complete diet to serve as replacements in case of accidents. When it was observed that 3 gm. of algae did not prevent the appearance of scurvy, the dosage was increased to 6 gm. Deaths from scurvy occurred in the animals fed algae as well as in the negative controls. Six gm. of algae are equivalent to 0.56 gm. of dried material, which is a small dose.

Discussion

These experiments indicate how certain plant processes and syntheses may be studied under known conditions by use of a plant behaving normally in darkness as well as in light and growing in pure culture under laboratory conditions. The failure of the plant to synthesize a particular vitamin does not necessarily signify that this organism is unable to synthesize the vitamin in nature, where a different nutrition may be available.

The results of the vitamin A experiment have a special interest, since the curative material was derived from a population long grown in complete darkness, for the inoculum per culture was not more than 5000 algal cells, and these multiplied in darkness until countless numbers of progeny resulted, which at no time during their growth had the added factor of illumination to be accounted for in the experiment. In the case of the etiolated seedlings, however, the seeds were produced in sunlight in the field. Also the hypothesis of SCHERTZ (**15**) that vitamin A is formed from pyrrole-ringed compounds found in soil cannot be substantiated, since none of the ingredients of our substance contained any substance remotely related to such compounds. Carotene was undoubtedly present (**10**) in the algae, however.

The positive results obtained in the experiments on vitamins B and G are not surprising. The organism belongs to the phylum Thallophyta.

comprising the algae and fungi. It is well recognized that fungi are able to synthesize large amounts of vitamin B in darkness, and it is also a commonly accepted but broad conception that fungi are algae devoid of chlorophyll. The organism used resembles a fungus in that it is able to exist saprophytically on organic media, and therefore fulfills an important attribute of a fungus but differs radically because of its power to manufacture chlorophyll in light or in darkness.

No data have been found relative to the vitamin C content of naturally occurring algae. It would be surprising if these plants in their natural environment did not synthesize this vitamin, which is universally present in succulent green plants.

Summary and conclusions

1. A unicellular green plant, *Chlorococcum* sp., synthesizing chlorophyll in complete darkness and able to exist saprophytically, was studied. Any synthesis of vitamins must of necessity have been elaborated by the cells without light, bacterial symbiosis, or complex nutrients. Such synthesis was effected from inorganic salts with a pure carbohydrate, dextrose as the only source of energy.

2. Vitamin A or its provitamin was shown to be synthesized in large quantities under these conditions. Dosages much smaller than those previously reported by workers using etiolated seedlings were more potent than the amount of seedlings fed.

3. Vitamins B and G were likewise found, but no vitamin C could be detected in the quantity of algae fed as supplement.

DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
UNIVERSITY OF MINNESOTA
MINNEAPOLIS, MINNESOTA

LITERATURE CITED

1. AHMAD, BASHIR. Observations on a diatom (*Nitzschia closterium* W. Sm.) as a source of vitamin A. Biochem. Jour. **24**: 860–869. 1930.
2. ARTARI, A. Über die Entwicklung der grünen Algen unter Ausschluss der Kohlensäure Assimilation. Bull. Soc. Imp. Nat. Moscou 39–47. 1889.
3. CHICK, HARRIETTE, and DELF, ELLEN M. The anti-scorbutic value of dry and germinated seeds. Biochem. Jour. **13**: 199–217. 1919.
4. COWARD, KATHERINE H. The influence of light and heat on the formation of vitamin A in plant tissues. Jour. Biol. Chem. **72**: 781–799. 1927.
5. FÜRST, V. Weitere Beiträge zur Ätiologie des experimentellen Skorbuts des Meerschweinchens. Zeitschr. Hyg. **72**: 121–154. 1912.

6. GUNDERSON, M. F., and SKINNER, C. E. Suggestions for growing mass cultures of algae for vitamin and other physiological study. Plant Physiol. **7**: 539–540. 1932.
7. HELLER, V. G. Vitamin synthesis in plants as affected by light source. Jour. Biol. Chem. **76**: 499–511. 1928.
8. HONEYWELL, EDNA M., and STEENBOCK, H. The synthesis of vitamin C by germination. Amer. Jour. Physiol. **70**: 322–332. 1924.
9. JAMESON, H. L., DRUMMOND, J. C., and COWARD, KATHARINE H. Synthesis of vitamin A by a marine diatom (*Nitzschia closterium* W. Sm.) growing in pure culture. Biochem. Jour. **16**: 482–485. 1922.
10. MEIER, FLORENCE ELISABETH. Recherches expérimentales sur la formation de la carotine chez les algues vertes unicellulaires et sur la production de la gelée chez un *Stichococcus* (*S. mesenteroides*). Bull. Soc. Bot. Genève **21** (2 sér.): 161–197. 1929.
11. MENDEL, LAFAYETTE B. Vitamin A. Jour. Amer. Med. Assoc. **98**: 1981–1987. 1932.
12. MOORE, T. Vitamin A formation in the etiolated wheat shoot. Biochem. Jour. **21**: 870–874. 1927.
13. NELSON, V. E., FULMER, E. I., and CESSNA, RUTH. The synthesis of water-soluble B by yeast. Jour. Biol. Chem. **46**: 77–81. 1921.
14. PALMER, L. S., and KENNEDY, CORNELIA. The fundamental food requirements for the growth of the rat. I. Growth on a simple diet of purified nutrients. Jour. Biol. Chem. **74**: 591–611. 1927.
15. SCHERTZ, F. M. The chloroplast pigments, their functions, and the probable relation of chlorophyll to the vitamines. Quart. Rev. Biol. **3**: 459–485. 1928.
16. SKINNER, C. E. Isolation in pure culture of green algae from soil by a simple technique. Plant Physiol. **7**: 533–537. 1932.
17. ————, and GARDNER, CLARA G. The utilization of nitrogenous organic compounds and sodium salts of organic acids by certain soil algae in darkness and in the light. Jour. Bact. **19**: 161–179. 1930.
18. ————, and GUNDERSON, M. F. Production of vitamin A by a species of *Corynebacterium*. Jour. Biol. Chem. **97**: 53–56. 1932.
19. SMITH, MAURICE I., and HENDRICK, E. G. Some nutrition experiments with brewers' yeast with especial reference to its value in supplementing certain deficiencies in experimental rations. Pub. Health Reports **41**: 201–207. 1926.
20. SUNDERLIN, GERTRUDE, and WERKMAN, C. H. Synthesis of vitamin B by microörganisms. Jour. Bact. **16**: 17–33. 1928.

PREPARATION OF AQUEOUS EXTRACTS OF SOLUBLE NITROGEN FROM PLANT TISSUES[1]

O. W. DAVIDSON, H. E. CLARK, AND J. W. SHIVE

Introduction

It has been shown by CHIBNALL (3), and by TOTTINGHAM *et al.* (9), that the drying of plant tissues before extraction changes the proportional distribution of nitrogen among the nitrogenous constituents. Accordingly, only aqueous extracts from fresh plant tissues have been used by CHIBNALL (2), VICKERY and PUCHER (10), NIGHTINGALE (7), and MURNEEK (6) in studies on the nitrogen metabolism of plants. Their results have shown that the various nitrogenous fractions included in the aqueous extracts are indicative of the course of nitrogen metabolism in plants.

The relative advantages of the solvents commonly employed in the preparation of plant extracts have been discussed by other investigators (4, 5). Hot alcohol used in concentrations of 50 to 80 per cent. has been recommended because it stops enzymatic action quickly, precipitates some or all of the proteins, and is a good solvent for the relatively simple nitrogenous compounds. APPLEMAN and MILLER (1) found that extracts of potato tubers prepared by the use of hot alcohol yielded practically the same amounts of non-protein nitrogen as did cold-water extracts. Hot water will destroy enzymes about as rapidly as will hot alcohol, however, and it is a slightly better solvent for the simple nitrogenous constituents than is the latter. By the use of hot water, cell membranes are killed quickly and thereby rendered permeable to the soluble constituents. Furthermore, the use of hot water simplifies the subsequent analytical procedure, since certain nitrogen determinations can be made only with alcohol-free samples (4).

In the preparation of aqueous extracts of soluble nitrogen, TOTTINGHAM *et al.* (9) and NIGHTINGALE (7) ground the plant tissues in a mortar with the aid of sand. The preparation of extracts by this method is attended with much labor and requires considerable time. This in turn limits the number of samples that can be extracted within a given period. Moreover, the time required for the preparation of an extract in this manner permits a loss of nitrogen or a change in the distribution of the nitrogenous fractions in plants containing such unstable compounds as some of the cyanogenetic glucosides (8).

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

It appears, therefore, that a method of extraction by boiling the tissue in water, somewhat similar to that used by VICKERY and PUCHER (10), would be rapid and might be applicable for use with plants containing unstable nitrogenous compounds. The development of such a method of extraction, and the testing of its applicability in comparison with the method now rather generally employed, have been the chief purposes of this investigation.

Finely minced plant tissue was divided into representative aliquots, some of which were boiled in water and then extracted, while others were ground in a mortar with sand before extraction. Quantitative comparisons were made of the commonly separable soluble nitrogenous fractions contained in samples prepared by the two methods.

Methods of extraction

In the preparation of an extract by the method recommended by TOTTINGHAM *et al.* (9), a 50-gm. or 100-gm. aliquot of finely minced or shredded plant material was ground in a large mortar with the aid of washed quartz sand, but without the use of ether as a plasmolyzing agent. The ground tissue was then transferred quantitatively on to a piece of "longcloth" and washed thoroughly with successive applications of water, each of which was removed by wringing with the hands. The aliquot was ground again in the mortar and then washed repeatedly on the same piece of cloth until the extract comprised a volume of about 950 cc. For convenience, this procedure for the preparation of extracts will be referred to as the grinding method.

The method of extraction by boiling is briefly as follows: A 50-gm. or a 100-gm. aliquot of finely minced plant tissue was placed in a beaker, covered with boiling water, and boiled on a hot plate for 20 minutes. The contents of the beaker were then transferred to an 18-inch square of longcloth, washed with successive applications of water, and extracted by wringing with the hands. After the tissue was washed four or five times, it was transferred to a mortar in which any large pieces of tissue were pounded to a pulp without the use of sand. This was easily accomplished, since the boiling treatment softens the tissue. The sample was then returned to the cloth directly, or, in the case of the cotton and peach samples, it was boiled for 10 minutes in a beaker and then transferred to the cloth. The washing and wringing were continued until the extract comprised a volume of 950 cc. This complete procedure will be referred to as the boiling method.

Peach stems are very woody and therefore were difficult to mince with a knife, but they were cut easily to shreds in a pencil sharpener. Short pieces that could not be cut in a pencil sharpener, and also the non-rigid, slightly lignified stem tips, were minced with pruning shears. The basal portions of nearly mature cotton stems are likewise woody, but they were

minced conveniently by the use of a large knife. Because of the woody and bulky nature of peach and cotton stems, it was thought advisable to boil these tissues for 10 minutes after they were pulped in a mortar. The need for the second boiling treatment has not been determined experimentally for plant materials so woody as these, but the data presented in tables II and III show that a second boiling treatment is unnecessary in the extraction of corn and tomato tissues.

The coagulable material, which was always proportionately small in amount when this method of extraction was followed, was precipitated by boiling the extract for one minute after the addition of 2 cc. of 10 per cent. acetic acid. The hot extract was then filtered through nitrogen-free filter pulp in a Buchner funnel. The filtrate was made to a volume of one liter and aliquots of this were used for the quantitative estimation of the nitrogenous fractions present.

Comparison of results

Duplicate 50 gm. samples of cotton and peach tissue were extracted by the two methods just described. A quantitative comparison of the nitrogenous constituents extracted from the samples by each method is shown in table I. It is evident from these comparisons that the two methods are equally efficient in removing the soluble nitrogenous constituents from the plant material.

TABLE I

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH COTTON AND PEACH TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	COTTON STEMS AND PETIOLES		PEACH STEMS	
	BOILING	GRINDING	BOILING	GRINDING
	%	%	%	%
Total N in tissues	0.339	0.339	0.504	0.504
Total soluble N	0.172	0.174	0.127	0.121
Amide N	0.025	0.025	0.013	0.013
Humin N	0.011	0.012	0.007	0.006
Basic-free amino N	0.035	0.035	0.015	0.016
Basic N	0.038	0.038	0.032	0.030
Cyanogenetic N			0.045	0.039
Ammonium N	0.008	0.008	0.013	0.014
Nitrate N	0.031	0.031	0.000	0.000

It is also evident that there is no difference in the nitrogenous composition of extracts prepared by these methods except in the case of the peach, which loses cyanogenetic nitrogen during the process of grinding. Such a

loss, however, may be anticipated when peach stems, leaves, or roots are ground and extracted for half an hour or more previous to a boiling treatment which stops enzymatic action. On the other hand, when peach tissue is minced quickly, covered immediately with boiling water and boiled for 20 minutes, no appreciable loss of this form of nitrogen takes place except in the case of rapidly growing twigs.

In order to obtain a further comparison of these methods of extraction, two representative 100-gm. aliquots of well minced corn stems were boiled for 20 minutes and then extracted as previously described. Two other aliquots from the same lot of stems were extracted by the grinding method. The extracts were analyzed, and the results are shown in table II. It is apparent that the boiling method, as well as the grinding method, gives consistent results when duplicate aliquots are extracted, and that variations in the composition of extracts from different samples are not due to the method of extraction. The data in table II also show that a single 20-minute period of boiling is adequate for the extraction of the soluble nitrogenous constituents from samples that are not very woody.

TABLE II

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH CORN TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	CORN STEMS				CORN LEAVES	
	BOILING		GRINDING			
	NUMBER OF ALIQUOT				BOILING	GRINDING
	A	B	C	D		
	%	%	%	%	%	%
Total N in tissues	0.174	0.174	0.174	0.174	0.705	0.705
Total soluble N	0.108	0.108	0.107	0.109	0.112	0.120
Amide N	0.002	0.003	0.003	0.003	0.002	0.002
Basic-free amino N	0.013	0.014	0.014	0.014	0.025	0.026
Basic N	0.017	0.017	0.016	0.015	0.037	0.039
Ammonium N	0.006	0.005	0.005	0.005	0.003	0.004
Nitrate N	0.077	0.076	0.075	0.075	0.051	0.050

In another test, triplicate aliquots were taken from a well mixed sample of finely minced tomato stems and leaves. One of the aliquots was extracted by the grinding method previously described. Another was boiled 20 minutes before extraction according to the procedure just outlined. The third aliquot was boiled for 5 minutes, extracted by washing and wringing several times, then returned to fresh boiling water and boiled again for 5 minutes. After a second extraction similar to the first, the boiling was repeated and the extraction completed by further washing and wringing. The results appear in table III and show that a single boiling

treatment for 20 minutes, followed by thorough washing and wringing, was just as effective in the removal of the soluble nitrogenous compounds from the tissue as was repeated boiling and extraction. Furthermore, the results show that both of the boiling methods of extraction were slightly more effective in the removal of soluble nitrogen than was the grinding method.

TABLE III

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH TOMATO TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	Composite of stems and leaves		
	Single boiling	Repeated boiling	Grinding
	%	%	%
Total N in tissues	0.447	0.447	0.447
Total soluble N	0.207	0.206	0.197
Amide N	0.006	0.006	0.005
Total amino N	0.036	0.035	0.035
Ammonium N	0.009	0.009	0.010
Nitrate N	0.103	0.101	0.098

From the results of this investigation it is evident that the preparation of aqueous extracts of the soluble nitrogenous constituents from plant tissues by the boiling method here described is equally as effective as is the preparation of these extracts by the grinding method (9) using quartz sand in a mortar, and is much more economical of time and labor. Furthermore, this method limits the possibility of a loss of nitrogen, and of a change in the composition of the extract with respect to the nitrogenous constituents.

Summary

1. Two methods for the preparation of aqueous extracts of the soluble nitrogenous constituents from plant tissues have been compared.

2. It was found that the preparation of extracts by a process of boiling for 20 minutes, followed by thorough washing through "longcloth," was just as effective in the removal of soluble nitrogenous constituents as was extraction by the process of grinding in a mortar with sand, followed by thorough washing through the cloth.

3. The preparation of aqueous extracts by the boiling method is easier and quicker than by the grinding method.

4. The loss of cyanogenetic nitrogen which takes place when peach extracts are prepared by the grinding method may be minimized by the use of the boiling method.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

1. APPLEMAN, C. O., and MILLER, E. V. A chemical and physiological study of maturity in potatoes. Jour. Agr. Res. **33**: 569–577. 1926.
2. CHIBNALL, A. C. Investigations on the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 344–362. 1922.
3. ————————. III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 597–609. 1922.
4. COMMITTEE ON METHODS OF CHEMICAL ANALYSIS. American Society of Plant Physiologists. The determination of nitrogen in relatively simple compounds. Plant Physiol. **2**: 205–211. 1927.
5. LOOMIS, W. E. Some problems in the analysis of horticultural material. Proc. Amer. Soc. Hort. Sci. **21**: 365–370. 1924.
6. MURNEEK, A. E. Effects of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon esculentum* Mill.). Plant Physiol. **1**: 3–56. 1926.
7. NIGHTINGALE, G. T. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.
8. ROBINSON, MURIEL E. Methods for the determination of nitrogenous constituents of a cyanophoric plant: *Prunus laurocerasus*. Biochem. Jour. **23**: 1099–1113. 1930.
9. TOTTINGHAM, W. E., SCHULZ, E. R., and LEPKOVSKY, S. The extraction of nitrogenous constituents from plant cells. Jour. Amer. Chem. Soc. **46**: 203–208. 1924.
10. VICKERY, H. B., and PUCHER, G. W. Chemical investigations of the tobacco plant. II. The chemical changes that occur during the curing of Connecticut shade-grown tobacco. Connecticut Agr. Exp. Sta. Bull. 324. 1931.

RELATION OF CERTAIN AMINO ACIDS TO CARBON DIOXIDE AND MYCELIUM PRODUCTION OF *FUSARIUM OXYSPORUM*

ARTHUR K. ANDERSON AND KATHRYN EMMART

(WITH ONE FIGURE)

Introduction

It has been known for many years that the ingestion of foods, especially proteins, stimulates metabolism in animals. LUSK (**11, 12**) has shown that certain amino acids produce the same stimulation as proteins. He believes that the stimulatory effect of proteins can be accounted for by the active amino acids which they contain. LUSK (**10**) has shown that glycine, alanine, leucine, and tyrosine stimulate oxidation in the order given, while glutamic acid does not. ATKINSON and LUSK (**3**) added aspartic acid and asparagin to the list of those which showed no effect. RAPPORT and BEARD (**15**) found phenylalanine to have the greatest stimulatory effect of any amino acid. ORT and BOLLMAN (**14**) found that cystine, glycine, alanine, phenylalanine, leucine, histidine, and valine catalyzed the action of hydrogen peroxide on dextrose, while glutamic acid, aspartic acid, and tyrosine had no effect.

With regard to the effect of amino acids on plant metabolism there is a considerable literature. In green plants SPOEHR and MCGEE (**17**) noted that the leaves of *Helianthus annuus* when fed a glycine solution consumed glucose more rapidly, and showed a greater respiratory activity as measured by carbon dioxide evolution, than leaves not so fed. KOSER (**9**) observed that certain amino acids supported growth of some bacteria but not others. GORDON and MCLEOD (**6**) noticed no marked effect of amino acids on hardy bacterial organisms such as *B. coli,* but that they stimulated the growth of certain delicate organisms. Several workers (**8, 13, 20**) have shown that certain amino acids influence the amount and size of yeast cells and also yeast activity in fermentation.

With regard to the effect of amino acids on fungi, SCHULZE (**16**) has shown that leucine and tryosine serve as a food material for *Penicillium glaucum.* TITS (**18**) found that leucine, glutamic acid, histidine, cystine, and glucosamine stimulated the germination of spores of *Phycomyces nitens.* HERZOG and SALADIN (**7**) have reported a decided stimulation in the metabolism of *Penicillium glaucum,* as measured by carbon dioxide production, after the addition of leucine to the medium.

In this laboratory considerable work has been done on the metabolism of *Fusarium oxysporum*. ANDERSON, EVERITT, and ADAMS (2) have shown that the main products of metabolism of this organism, when grown in a medium in which glucose is the only source of carbon, are carbon dioxide and ethyl alcohol. Since *Fusarium oxysporum* grows well in a medium containing no organic nitrogen, it was felt that this organism offered an excellent opportunity to study the stimulatory effect of amino acids on fungus metabolism. This paper reports a study of the rate of metabolism of *F. oxysporum* in glucose media containing glycine, leucine, tyrosine, and aspartic acid as measured by carbon dioxide production. Glycine, leucine, and tyrosine were selected as amino acids which might be expected to produce a stimulation in metabolism and the aspartic acid as one which might not.

Methods

The culture of *Fusarium oxysporum* used was obtained from the Division of Plant Pathology at the University of Minnesota. The stock medium was that used by ANDERSON (1) in his work on *F. lini* and had the following composition:

Ammonium nitrate	1.00 gm.
Monopotassium phosphate	0.50 "
Magnesium sulphate	0.25 "
Glucose	20.00 "
Water to make	1000 cc.

Using this as a basis, the amino acid media were prepared by adding 0.5 gm. carbon equivalent of an acid for each 300 cc. of stock medium (the amount used in each culture flask), *i.e.*, 1.5635 gm. of glycine, 0.9105 gm. of leucine, 0.8385 gm. of tyrosine, and 1.386 gm. of aspartic acid. Two controls were run, control A being the stock solution alone and control B being the same as control A except that 0.5 gm. of carbon in the form of glucose (1.25 gm.) was added to each flask. Three hundred cc. of each medium were placed in 500-cc. pyrex Erlenmeyer flasks. Triplicate experiments were run in each case. Each flask was closed by a two-hole rubber stopper through which extended two glass tubes. Cotton plugs were placed in the outer end of each tube to prevent contamination.

After autoclaving at 15 pounds' pressure for 20 minutes and cooling, each flask was inoculated with 10 cc. of spore and mycelium suspension. The stoppers were sealed with paraffin and the ends of the glass tubes were closed with rubber tubes and screw clamps.

Separate samples of all solutions were autoclaved with the culture flasks and, after cooling, their pH values were determined by the electrometric

method using a quinhydrone electrode. During the experiment the flasks were kept on laboratory desks at room temperature.

Carbon dioxide determinations were made at frequent intervals by aeration into TRUOG (19) towers containing barium hydroxide solution. The excess barium hydroxide was then titrated with 0.1 N hydrochloric acid using phenolphthalein as an indicator.

The cultures were allowed to grow for 229 days, at which time two sets, those containing glycine and aspartic acid, were opened as it was planned to terminate the experiment at that time However, the others were then allowed to grow longer, 270 days in all. After opening, the cultures were immediately filtered through Gooch crucibles and the mycelia washed, the washings being discarded. The mycelia were dried to constant weight at 100° C. The filtrates were used for the determination of pH and of residual glucose by the method of FOLIN and WU (5).

Presentation of data

Figure 1 and table I present the results of this investigation. Figure 1 shows the total amount of carbon dioxide produced during growth. This amount is the average of triplicate determinations for each culture medium.

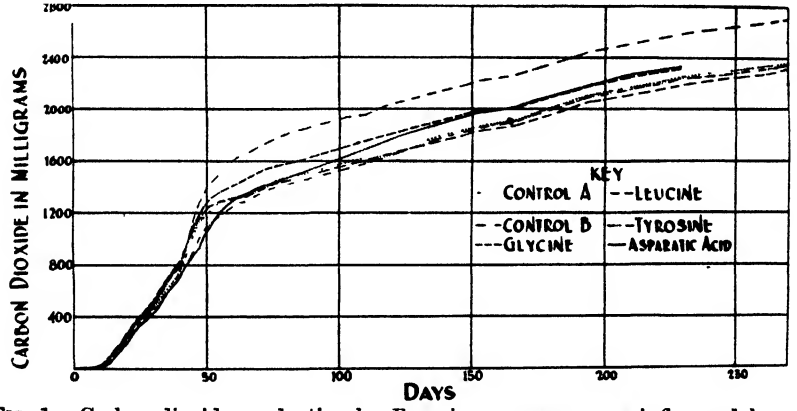


FIG. 1. Carbon dioxide production by *Fusarium oxysporum* as influenced by certain amino acids.

After a determination, the weight obtained from each culture was added to that previously obtained, so that the amount given for any day represents the total carbon dioxide produced from the time of inoculation to that day. It can be observed that control B, the medium modified by the addition of glucose, produces the greatest amount of carbon dioxide, which indicates that the carbon in glucose is the best source for carbon dioxide production by the fungus. The curve for the carbon dioxide produced by the fungus on the medium modified by aspartic acid is below all the others until the

53rd day, after which it gradually rises until on the 229th day it is above all except that for control B. The high initial acidity of this medium evidently causes the early retardation of metabolism, and, as the pH rises, a more optimum condition is reached, thereby producing a greater activity.

It can be observed that the glycine, as well as the aspartic acid medium, gave more carbon dioxide than control A. These two amino acids are therefore used as a carbon source by *Fusarium oxysporum*, although not to the same extent as an equal carbon equivalent of glucose. Tyrosine slows the rate of carbon dioxide production, while leucine has the greatest retarding effect. The comparisons are best made at 229 days, but by observing the chart one can see that the same ratio would exist if the curves for glycine and aspartic acid had been continued.

Table I gives a résumé of this investigation. The figures given represent the averages of triplicate determinations. It is unfortunate that the period of growth of the cultures containing glycine and aspartic acid was not so long as that of the others. It is therefore difficult to make comparisons. One can observe that the weights of mycelia obtained from the two cultures are even greater than that from control B in which growth was continued over a longer period of time. This might be accounted for by the beginning of autolysis when the fungus is allowed to grow after most of the glucose is consumed. On the other hand, since tyrosine produces a slightly greater weight than control B and leucine only a slightly smaller one, it appears that the amino acids studied, with the exception of leucine, are utilized to a greater extent than an equal carbon equivalent of glucose to produce fungus body.

The small amounts of residual glucose noted in the table show that most of the 6 gm. of glucose originally present in each flask is utilized during growth. The high values obtained for the cultures containing glycine and tyrosine are not significant because these two amino acids were found to have a slight reducing action on the reagents used in the glucose determination.

In every culture the hydrogen-ion concentration decreases during growth. The lowest original pH is for the media containing aspartic acid. This is due to the fact that this amino acid is a dicarboxylic acid. For the other media, the range of pH is from 4.6 for control B to 5.3 for that containing tyrosine. The final pH of all the media is 7.5 or above. Comparisons are again rather difficult on account of the difference in length of the period of growth of the cultures. The greatest increase in pH is noted for the medium containing aspartic acid, a change from 3.1 to 8.0. The medium containing glycine had a final pH of 8.1. As previously stated, these two amino acids, although the fungus was allowed to grow on them for a shorter length of time, produce the greatest weight of mycelium, and

TABLE I

TOTAL CARBON DIOXIDE AND MYCELIUM PRODUCTION, RESIDUAL GLUCOSE, AND ORIGINAL AND FINAL pH OF THE MEDIA

DESCRIPTION OF MEDIA		PERIOD OF GROWTH	WEIGHT			pH	
			CARBON DIOXIDE	DRY MATTER IN MYCELIUM	RESIDUAL GLUCOSE	ORIGINAL	FINAL
			mg.	*mg.*	*mg.*		
Controls	A: stock medium alone	270	2366.0	376.3	12.7	4.7	7.7
	B: stock medium plus glucose	270	2713.6	406.3	20.6	4.6	7.6
Media modified by addition of amino acids	Glycine	229	2322.4	483.8	37.6	4.8	8.1
	l-leucine	270	2321.9	403.1	28.8	4.9	7.7
	l-tyrosine	270	2351.6	410.9	67.1	5.3	7.5
	l-aspartic acid	229	2341.0	563.0	20.0	3.1	8.0

up to the time of the opening of the cultures, the greatest amount of carbon dioxide with the exception of control B.

Conclusions

At the outset of this experiment it was expected that certain amino acids which are known to produce a stimulation of metabolism in animals might do so with the fungus, *Fusarium oxysporum*. It is apparent from figure 1 that there is no such stimulatory effect as measured by carbon dioxide production. Cultures containing glucose in amounts equivalent in carbon content to the carbon of the added amino acids show just as rapid a production of carbon dioxide as those containing amino acids. In the end cultures containing added glucose produce much more carbon dioxide than do any of the cultures containing amino acids. Aspartic acid and glycine are utilized by the fungus for the production of carbon dioxide, while leucine and tyrosine retard the production of carbon dioxide.

All of the amino acids studied are utilized by the fungus in the production of mycelium. With the exception of leucine they are all a better source of mycelium building material than glucose. Aspartic acid is outstanding in its ability to produce mycelium.

PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PA.

LITERATURE CITED

1. ANDERSON, A. K. Biochemistry of plant diseases. VI. The biochemistry of *Fusarium lini* Bolley. Minnesota Studies Plant Sci. **5**: 237–280. 1924.
2. ————————, EVERITT, EDWARD L., and ADAMS, PHILIP D. The carbon metabolism of *Fusarium oxysporum* on glucose. Jour. Agr. Res. **46**: 473–482. 1933.
3. ATKINSON, H. V., and LUSK, GRAHAM. Animal calorimetry. XV. Further experiments relative to the cause of the specific dynamic action of protein. Jour. Biol. Chem. **36**: 415–427. 1918.
4. BAESTIE, R. Yeast growth stimulants. Wochenschr. Brau. **41**: 251–253. 1924. (Chem. Abst. **19**: 2220. 1925.)
5. FOLIN, O., and WU, H. A system of blood analysis. Supplement I. A simplified and improved method for determination of sugar. Jour. Biol. Chem. **41**: 367–374. 1920.
6. GORDON, J., and MCLEOD, J. W. Inhibition of bacterial growth by some amino acids and its bearing on the use of tryptic digests as culture media. Jour. Path. Bact. **29**: 13–25. 1926.
7. HERZOG, R. O., and SALADIN, O. Über das Verhalten einiger Pilze gegen Aminosäuren. Zeitschr. physiol. Chem. **73**: 302–307. 1911.

8. HOLT, MARY. Giant yeast cells. Trans. Roy. Soc. Canada **22**: 269–270. 1928.
9. KOSER, STEWART A. Studies upon bacterial nutrition. The utilization of nitrogenous compounds of definite chemical composition. Abstracts Bact. **2**: 12. 1918.
10. LUSK, GRAHAM. Animal calorimetry. V. The influence of the ingestion of amino-acids upon metabolism. Jour. Biol. Chem. **13**: 155–183. 1912.
11. ————————. The elements of the science of nutrition. W. B. Saunders Co., Philadelphia. 1928.
12. ————————. The specific dynamic action. Jour. Nutrition **3**: 519–530. 1931.
13. MACDONALD, MARGARET B., and MCCOLLUM, E. V. The cultivation of yeast in solutions of purified nutrients. Jour. Biol. Chem. **45**: 307–311. 1921.
14. ORT, J. M., and BOLLMAN, JESSE L. The catalytic and specific dynamic actions of certain amino acids. Jour. Amer. Chem. Soc. **49**: 805–810. 1927.
15. RAPPORT, D., and BEARD, H. H. The effects of protein split-products upon metabolism. II. The individual amino acids of fraction I of the butyl alcohol extraction, and their relation to the specific dynamic action of protein. Jour. Biol. Chem. **73**: 299–319. 1927.
16. SCHULZE, E. Können Leucin und Tyrosin den Pflanzen als Nährstoffe dienen? Landw. Vers.-Sta. **56**: 97–106. 1902.
17. SPOEHR, H. A., and MCGEE, J. M. Studies in plant respiration and photosynthesis. Carnegie Inst. Washington. Publication no. 325. 1923.
18. TITS, DÉSIRÉ. L'action des amino acids sur la germination de *Phycomyces nitens* Kunze et Schmidt. Bull. Sci. Acad. Roy. Belg. [5] **12**: 545–553. 1926.
19. TRUOG, E. Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. Ind. & Eng. Chem. **7**: 1045–1049. 1915.
20. ZELLER, HEINRICH. Wirkung N-haltiger Substanzen auf die Hefegärung. V. Biochem. Zeitschr. **176**: 134–141. 1926.

RESPIRATORY RATE AND ENZYME ACTIVITY AS RELATED TO THE HARDENED CONDITION OF PLANTS[1]

S. T. DEXTER[2]

(WITH ONE FIGURE)

Introduction

In several papers in the literature (**8, 3**), reference is made to the dormancy of plants or their rate of respiration, in connection with the hardened condition. ÄKERMAN (**1**) has shown that hardy varieties of winter wheat tend to retain larger stores of sugars throughout the winter than tenderer ones. NEWTON and ANDERSON (**6**) have followed the rate of respiration of several varieties of winter wheat throughout the growing season and found that the respiratory rate of each variety decreased as the season progressed, with the hardiest variety showing the lowest output of carbon dioxide per gram of dry matter. The implication in at least some of this work is that the hardened condition is accompanied by a lowered rate of respiration and "dormancy." NEWTON and BROWN (**7**) have studied the activity of catalase in the expressed sap of winter wheat varieties, but their results were difficult to interpret and highly variable.

Since it is possible to grow plants in such a way that hardening will not occur at a low temperature in the dark (**4, 9**), and equally possible to grow them so that hardening will occur, it seemed desirable to measure the carbon dioxide output from samples of plants so stored, and to determine the catalase and oxidase activity of the plants before and after exposure to the low temperature.

Methods

Duplicate samples (of about 15 plants each) of winter wheat from sand cultures were placed in flasks on damp asbestos fiber. Tests by the freezing-exosmosis method of DEXTER *et al.* (**4**) on plants from the same pots showed that hardening did not occur in the cold room (2° C.) in the dark. The flasks were stored in a room kept constantly at 2° C. for 24 hours before the measurements of carbon dioxide output were started. Compressed air was passed through a soda-lime tube, and through barium hydroxide solution, thence into the flasks, and into modified Pettenkoffer tubes containing tenth-normal barium hydroxide. Before the measurements the flasks were flushed out with carbon dioxide-free air. The passage of air through the

[1] Contribution from the Plant Physiology Laboratories, University Farm, University of Minnesota.

[2] National Research Council Fellow.

flasks was at a rate of about 15 cc. per minute, and was kept constant throughout the experiment by means of a bubble-counter. The blank on the apparatus for a run of 24 hours was 0.3 cc. of base neutralized. The total output of carbon dioxide from the plants in 24 hours at 2° C. was measured at the intervals indicated in table I. At the expiration of the experiment, the two samples were dried to constant weight, ashed, and the results computed on the basis of milligrams of carbon dioxide output per gram of organic matter in the sample. The experiment was repeated on another set of winter wheat plants from the same variety (Minhardi), and these results are included in the table. According to tests, plants from this second set did not harden in the dark at 2° C. In a similar way, the experiment was run with cabbage plants which were high in starch and which were shown to harden markedly during exposure to the low temperature.

Plants from the same sets of pots were tested for catalase and oxidase activity according to the following procedure. Before making the first analysis for catalase or oxidase, all plants were held in darkness at 2° C. for 24 hours. They were then divided into two sets, one of which was given continuous illumination, the other held in darkness. The cabbage plants hardened excellently whether in darkness or in light. The two sets of winter wheat failed to harden in darkness but hardened well in the light. After a period in the cold room, all samples were again held in darkness for 24 hours before samples were taken, in order to bring them to a condition relatively comparable to that at the beginning of the experiment. Each sample of winter wheat consisted of six plants, two from each of three pots. Cabbage samples consisted of 10 discs, 1 cm. in diameter, from one side of the leaves of an individual plant. After a period in the cold room, corresponding punches were made on opposite sides of the leaves. The determination of catalase followed that of LANDON (5). The green weight of the samples was determined, an equal weight of precipitated chalk was added (in the case of the wheat samples an arbitrary weight, 0.5 gm.), and the samples were quickly ground, with the addition of a pinch of quartz and a few drops of distilled water, to a smooth paste. The sample was then further diluted with water until fifty times the weight of the sample had been added. This aqueous suspension was stirred with an electric agitator while aliquots were withdrawn. Two cc. of sample were used in each determination of catalase. Duplicates rarely differed as much as 5 per cent. and usually were identical. Five cc. of solution were used in the determination of oxidase, with 10 cc. of 2.5 per cent. hydroquinone.

Table I gives the amount of CO_2 respired in 24 hours by 1 gm. of ash-free plant substance in the dark at 2° C. over a period of several days. This shows that there was a continuous decrease in respiration in each of the five samples. This seems somewhat remarkable in that the physiological condi-

TABLE I

RESPIRATORY RATE PER GRAM ASH-FREE DRY MATTER DURING CONTINUOUS EXPOSURE AT 2° C. FOR SEVERAL DAYS. VALUES ARE IN MILLIGRAMS CO_2 IN 24 HOURS

DAYS AT 2° C. BEFORE TESTED	SAMPLE				
	WHEAT 1 NON-VEGETATIVE (−N)		WHEAT 2 VEGETATIVE (+N)		CABBAGE
	a	b	a	b	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	17.68		7.36		15.88
2					8.37
3		8.14			7.41
4				7.42	5.82
5					5.19
6	6.53				4.07
7			5.53		3.96
8		5.39			3.71
9				5.40	
10	5.56				
11					
12					
13			5.37		
14				4.96	

tions of the plants were very different. Sample 1, wheat, was very non-vegetative (−N), since nitrogen had been withheld for a period in the greenhouse. It contained no starch, but was high in sugars. It did not harden in the cold room. Sample 2, wheat, was highly vegetative (+N), and also failed to harden in the cold room in the dark. The cabbage sample was high in starch and relatively low in sugar at the beginning of the experiment, according to analysis on similar tissue, and at the end of the experiment was decidedly higher in sugar (about 30 per cent.) and lower in starch. Similar cabbage plants hardened well under the conditions of the experiment. In analyses of similar wheat samples, it was found that the sugar decreased materially during storage in the dark, whereas it increased in the case of the cabbage. No essential difference is evident in the trend of the respiration in either case. Furthermore, whether the plants hardened

or failed to harden, and whether the sugar percentage increased or decreased, a similar repression of respiration during continuous storage at the low temperature is most evident. BARKER (2) has recently noted a similar depression in the rate in respiration during long-continued storage of potatoes at a low temperature, even though the percentage of sugar was increasing greatly.

Figure 1 presents the data of table I in graphic form.

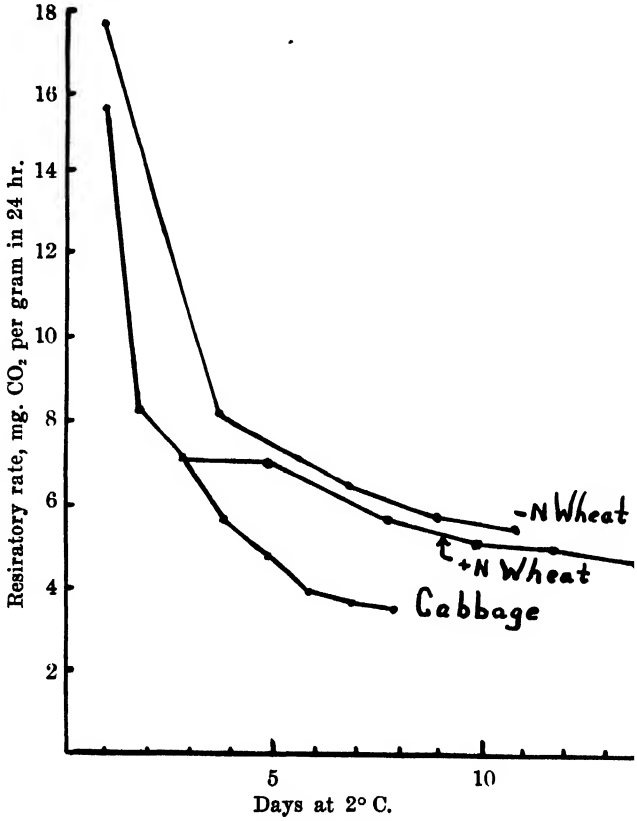


FIG. 1. Data of table I in graphic form: Respiratory rate of all samples decreased over a period of several days at 2° C. regardless of hardening or increase in sugars.

In table II are given the results of the determinations of catalase activity in cabbage and winter wheat plants which were subjected to continuous cold. The value for catalase is the volume of oxygen set free in 5 minutes. The oxidase activity was determined on the same samples. The average change in oxidase activity during exposure for 5 days to 2° C. for the first four samples of cabbage was from 3.0 cc. to 2.8 cc. For the second set of

four samples, from 3.1 cc. to 3.0 cc. This change is less than the blank with distilled water. The changes with winter wheat were, similarly, within the experimental error.

TABLE II

CATALASE ACTIVITY OF CABBAGE AND WINTER WHEAT PLANTS BEFORE AND AFTER EXPOSURE TO LOW TEMPERATURES (2° C.). VALUES ARE IN CC. OF OXYGEN LIBERATED FROM HYDROGEN PEROXIDE IN 3 MINUTES BY A SAMPLE OF 0.04 GM. GREEN WEIGHT

DAYS AT 2° C. BEFORE TESTED	OXYGEN SET FREE IN 5 MINUTES' REACTION WITH HYDROGEN PEROXIDE							
	CABBAGE PLANTS				WHEAT PLANTS			
	IN LIGHT		IN DARK		IN LIGHT		IN DARK	
	+N	−N	+N	−N	+N	−N	+N	−N
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	4.4	5.0	3.8	7.2	11.7	10.2	11.8	11.3
6	4.5	4.7	4.6	7.4	8.9	9.2	13.4	15.3
11	.....	.....	.....	.....	7.4	8.6	15.5	18.3
	Repeated all dark							
1	7.8	8.6	8.8	10.0	....	.....	.....	.. ..
6	7.4	9.1	9.5	9.1	.....	.....	.....	.....

From table II it would appear that the catalase activity of cabbage plants does not change perceptibly in either the light or the dark as a result of exposure to continued cold, during which time marked increase in resistance to freezing injury has developed. The change in the first four samples was from 5.1 to 5.3 cc.; in the case of the second set of samples, the averages were 8.8 and 8.78 cc.

The winter wheat plants which received continuous light, thereby increasing markedly in percentage dry matter and in hardiness, showed an unmistakable decrease in catalase activity. Those plants which were stored in the dark showed an increase in catalase activity. If the values were computed on a dry matter basis, rather than on a green weight basis, these differences would be still more pronounced. There is an evident difference therefore in the behavior of the two species used, since the cabbage showed virtually no change in catalase activity in either case. There was, however, a marked increase in percentage dry matter in the case of the wheat which was illuminated during storage. This relatively large amount of sugar and other storage material might be low in catalase. The cabbages, to the contrary, were already high in dry matter, and heavily stored with starch when put in the cold room. At the end of the experiment there happened to be a

slightly greater dry matter content in those cabbages which were stored in the dark than in those which had received light. Probably not much photosynthesis took place in these already high-carbohydrate plants.

There is a notable lack of correlation between change in respiratory rate and catalase or oxidase activity during exposure to low temperatures. For, although respiratory rates decreased during storage at 2° C. in vegetative and non-vegetative wheat plants and in high-carbohydrate cabbage plants, the oxidase activity of none of them changed perceptibly during this period. The catalase activity of both types of wheat increased (in the dark) while that of the cabbage plants remained constant. The lack of correlation between change in respiratory rate and change in hardiness is equally noticeable. In all cases, whether increase in hardiness took place or not, a seemingly identical behavior was noted in the decreasing respiration of the samples during the exposure at 2° C. If the same mechanism is at work here that BARKER (**2**) has described in the case of potatoes (which do not harden materially), it would appear that a "respiratory depressant" is produced regardless of increase in sugar or increase in hardiness.

Summary and conclusions

1. Cabbage and winter wheat plants were examined for rate of respiration and catalase and oxidase activity during exposure to a low temperature (2° C.).

2. Respiratory rates of all samples continuously decreased during storage at this temperature, regardless of increase in percentage sugar in the tissues or increase in hardiness.

3. Oxidase activity appeared unchanged as a result of such storage, and was not seemingly correlated with rate of respiration, with increase in sugars, or with change in hardiness.

4. Catalase activity in cabbage was not changed by storage at 2° C. for five days, either in the light or in the dark, although in both cases the plants hardened markedly and showed large increases in percentage of sugars.

5. Catalase activity decreased in winter wheat plants which were illuminated in the cold room, and increased in those stored in the dark. According to the interpretation of the writer, this change should not be associated with the differences in hardiness which developed under those two conditions of storage. It seems more likely that the increase of storage material in the plants in the light may be responsible for the decrease in catalase activity, since storage tissue in the crown was found much lower in catalase activity than leaf-blade tissue on the same plants.

6. Although hardy varieties of winter wheat probably have a lower respiratory rate than tender varieties (**6, 1**), we have no evidence to show that the hardened condition as such is especially characterized by a low

respiratory rate. Plants which have been stored at a low temperature appear to suffer great depressions in respiratory rates whether they harden or not.

7. It seems necessary to correlate more clearly our ideas of dormancy, rest period, and cold resistance of plants.

UNIVERSITY FARM
ST. PAUL, MINNESOTA

LITERATURE CITED

1. ÅKERMAN, Å. Studien über den Kaltetod und die Kalteresistenz der Pflanzen nebst Untersuchen über die Winterfestigkeit des Weizens. 1–232. Lund. 1927.
2. BARKER, J. Analytic studies in plant respiration. IV and V. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Proc. Roy. Soc. London B. **112**: 316–358. 1933.
3. BEAUMONT, J. H., and WILLAMAN, J. J. Preliminary report on the respiration of apple twigs during the winter. Proc. Amer. Soc. Hort. Sci. **21**: 99–104. 1924.
4. DEXTER, S. T. Effect of several environmental factors on the hardening of plants. Plant Physiol. **8**: 123–139. 1933. (See also, Plant Physiol. **5**: 215–223. 1930.)
5. LANDON, R. H. The effect of certain chemicals on the catalase activity of plants. Thesis. University of Minnesota. 1932.
6. NEWTON, R., and ANDERSON, J. A. Respiration of winter wheat plants at low temperatures. Canadian Jour. Res. **5**: 337–354. 1931.
7. ————————, and BROWN, W. R. Catalase activity of wheat leaf juice in relation to frost resistance. Canadian Jour. Res. **5**: 333–336. 1931.
8. STRAUSBAUGH, P. D. Dormancy and hardiness in the plum. Bot. Gaz. **71**: 337–357. 1921.
9. TUMANOV, J. J. Das Abhärten winterannueller Pflanzen gegen niedrige Temperaturen. Phytopath. Zeit. **3**: 303–334. 1931.

METHOD FOR THE PREPARATION OF GREEN PLANT MATERIAL FOR THE EXTRACTION OF JUICES[1]

L. D. DONEEN

Introduction

The extraction of plant juices is often desirable or essential for a study of their physico-chemical properties. As methods in general use are laborious and time consuming, it was found desirable in a recent investigation on the nutrition of wheat to develop a rapid, simple, yet accurate method for extracting the plant juice.

A review of the literature indicated that several methods have been devised for the treating of plant tissue to facilitate the expression of its juice. These methods may be divided into two general groups: either the material is ground in a mortar or grinding mill, or it is frozen in a slushy mixture of ice and salt, a cold storage room, liquid air, or solid carbon dioxide. The method in most frequent use is freezing the tissue in a test tube or jar for a number of hours, thawing it rapidly, and subjecting it to pressure. Some excellent and comprehensive reviews of the methods employed have been contributed by MEYER (**6**), GORTNER (**1**), SAYRE and MORRIS (**8**), and GREATHOUSE (**3**).

In the proposed method the material was heated in an autoclave. Approximately 100 young winter wheat plants were used, some grown in the greenhouse and others under natural field conditions of late winter and early spring. Juices were satisfactorily obtained from the wheat plants at regular intervals during the growing period.

Procedure

The wheat plants were wrapped in damp cloths in the field, placed in a moist, air-tight container, and brought immediately to the laboratory. The wrapped plants were then placed in an autoclave for five minutes at 15 pounds' pressure, although a reasonable variation in the length of time does not affect the results. Material which was not wrapped tightly was often sufficiently cooked in one minute at 15 pounds' pressure, but it was found advisable to leave the tightly wrapped material in the autoclave for five minutes or until the thoroughly cooked plant tissue was light brown in color. After autoclaving, the sample was removed and cooled to room temperature. According to the results of a number of trials, the moisture content of the tissue does not seem to be affected materially by autoclaving.

[1] Published as Scientific Paper no. 278, College of Agriculture and Experiment Station, State College of Washington.

The small amount of condensation that may occur during the heating was lost by evaporation while cooling to room temperature. The sap was expressed as soon as the material was cool to prevent further loss of moisture and possible changes in percentage of the plant juice.

A large percentage of the juice might have been expressed by hand, but to be certain of obtaining a representative sample, a hydraulic press was employed, applying uniform pressures of 7000 pounds per square inch and uniform times of draining of four minutes after this pressure was reached. Approximately 90 per cent. of the plant juices was extracted by this method. However, this varies with the type of plant, its age, and the conditions under which it is grown.

The method of freezing the wheat plants in a cold storage room at a temperature of 30° C., and the method of autoclaving them antecedent to expression of the sap were compared for determinations of the freezing point, total solids, total sugars, and nitrate-nitrogen of the expressed plant juice.

The freezing point of the juice was determined in the usual freezing point apparatus with a Beckmann thermometer. The figures reported were the results of four closely agreeing determinations on each sample. The freezing point depression readings were converted into equivalent osmotic values according to the equation of LEWIS (5).

The percentage total solids in the expressed plant juice was obtained by the Abbé refactometer according to GORTNER and HOFFMAN (2).

The total sugar content was determined by the Munson-Walker method, the cuprous oxide being weighed directly according to the recommendation of the Association of Official Agricultural Chemists (7).

The nitrate-nitrogen content was found by the colorimeter method of HOLTZ and LARSON (4).

Results

A comparison of the results for a number of determinations of osmotic pressure and total solids, obtained by the proposed autoclave method and freezing method antecedent to expressing the plant juice, is given in table I.

Samples 1, 2, 3, 4, and 5 were grown in the greenhouse during the winter. Samples 6 and 7 were grown on land continuously cropped by wheat and were sampled in the field in the early spring. Samples 8 and 9 were similar to samples 6 and 7 except that they were grown on summer fallow land.

A comparison of the total sugar and nitrate-nitrogen of the expressed juice as obtained by the two methods is given in table II.

The osmotic pressure, total solids, total sugar, and nitrate-nitrogen of the autoclaved and frozen plant tissue agree closely. The small variation

TABLE I

OSMOTIC PRESSURE AND TOTAL SOLIDS OF PLANT SAP OBTAINED BY AUTOCLAVING AND BY FREEZING THE PLANT MATERIAL ANTECEDENT TO EXPRESSING THE SAP

Sample no.	Wheat variety	Method of treatment	Freezing point depression	Osmotic pressure		Total solids	
				Atmospheres	Difference	Percentage	Difference
			° C.				
1	Hybrid 128	Frozen	1.062	12.78	− 0.10	7.7	− 0.20
		Autoclaved	1.054	12.68		7.5	
2	White Odessa	Frozen	0.908	10.96	− 0.11	5.4	+ 0.30
		Autoclaved	0.900	10.85		5.7	
3	Coppie	Frozen	1.120	13.48	− 0.25	6.25	+ 0.35
		Autoclaved	1.099	13.23		6.6	
4	Hussar	Frozen	0.940	11.32	+ 0.55	5.25	− 0.20
		Autoclaved	0.986	11.87		5.05	
5	Hybrid 128	Frozen	0.953	11.48	− 0.19	4.90	+ 0.15
		Autoclaved	0.938	11.29		5.05	
6	Ruddy	Frozen	0.861	10.37	+ 0.11	9.25	− 0.10
		Autoclaved	0.870	10.48		9.15	
7	Hybrid 128	Frozen	0.800	9.60	0.00	8.80	− 0.30
		Autoclaved	0.802	9.60		8.50	
8	Ruddy	Frozen	0.688	8.28	+ 0.16	5.70	+ 0.05
		Autoclaved	0.701	8.44		5.75	
9	Hybrid 128	Frozen	0.658	7.91	− 0.21	5.40	0.00
		Autoclaved	0.648	7.80		5.40	

that occurred could be accounted for partly by the difference in sampling. The proposed treatment of plant tissue can therefore be satisfactorily used for some phases of plant juice study instead of the freezing method.

SAYRE and MORRIS (8) showed that the sugar content of tissue could be calculated from the sugar content of the expressed juice. A comparison of the percentage sugar and nitrate-nitrogen in the plant juice from the autoclaved material with that of the dried wheat plant is given in table III.

The tissue was dried at a temperature of 60° C. in an air current. There were smaller differences between the duplicate autoclaved samples than between samples of dried tissue. This is especially true of sugar content.

Numerous investigations have shown that drying of green plant material has a tendency to result in a change of the various carbohydrates and proteins. This is well illustrated in table III.

The autoclave method cannot be used in a study of proteins, that is, proteins precipitated by heat, any more than the freezing method can be used in such a study. This method, however, has many advantages for the study of the truly soluble material not affected by heat. For instance, the

TABLE II

TOTAL SUGAR AND NITRATE-NITROGEN OF PLANT SAP OBTAINED BY AUTOCLAVING AND BY FREEZING THE PLANT MATERIAL ANTECEDENT TO EXPRESSING THE SAP

SAMPLE NO.	WHEAT VARIETY	METHOD OF TREATMENT	TOTAL SUGAR		NITRATE-NITROGEN	
			PERCENTAGE	DIFFERENCE	PERCENTAGE	DIFFERENCE
			%		%	
3	Coppie	Frozen	0.41	−0.02	0.157	−0.004
		Autoclaved	0.39		0.153	
5	Hybrid 128	Frozen	0.41	+0.01	0.141	+0.004
		Autoclaved	0.42		0.145	
6	Ruddy	Frozen	3.75	−0.10	0.018	+0.001
		Autoclaved	3.65		0.019	
7	Hybrid 128	Frozen	3.60	+0.05	0.009	+0.002
		Autoclaved	3.65		0.011	
8	Ruddy	Frozen	0.78	+0.04	0.041	−0.003
		Autoclaved	0.82		0.038	
9	Hybrid 128	Frozen	0.65	+0.03	0.032	−0.002
		Autoclaved	0.68		0.030	

enzyme action is almost instantly stopped by the high temperature in the autoclave. A large proportion of the plant juice is extracted and consequently a more representative sample of plant sap is obtained. This is apparently due to a better breaking down of the membranes and cell walls by the heat. The method is rapid as it is possible to autoclave and express ten samples of plant tissue per hour.

TABLE III

PLANT SAP AND DRIED WHEAT TISSUE COMPARED FOR DETERMINATIONS OF TOTAL SUGAR AND NITRATE-NITROGEN BASED ON DRY WEIGHT

VARIETY	PLANT TREATMENT	PERCENTAGE SUGAR			PERCENTAGE NO_3–N		
		SAMPLE		DIFFERENCE	SAMPLE		DIFFERENCE
		1	2		1	2	
Ruddy	Sap autoclaved	6.02	6.11	0.09	1.02	0.98	0.04
	Tissue dried	6.33	5.60	0.73	0.88	0.92	0.04
Little Club	Sap autoclaved	4.88	4.88	0.00	1.04	1.01	0.03
	Tissue dried	4.88	3.17	1.71	0.90	1.10	0.20
Jones Fife	Sap autoclaved	7.35	6.93	0.42	0.86	0.86	0.00
	Tissue dried	6.02	4.88	1.14	1.04	1.02	0.02

STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

LITERATURE CITED

1. GORTNER, R. A. Outlines of biochemistry. John Wiley and Sons, New York. 1929.
2. ————————, and HOFFMAN, W. F. Determination of moisture content of expressed plant tissue fluids. Bot. Gaz. **74**: 308–313. 1922.
3. GREATHOUSE, G. A. Effects of the physical environment on the physicochemical properties of plant saps, and the relation of these properties to leaf temperature. Plant Physiol. **7**: 349–390. 1932.
4. HOLTZ, H. F., and LARSON, CARL. A simple method for nitrate nitrogen determination in wheat plants. Plant Physiol. **4**: 288–291. 1929.
5. LEWIS, G. N. The osmotic pressure of concentrated solutions, and the laws of the perfect solution. Jour. Amer. Chem. Soc. **30**: 668–683. 1908.
6. MEYER, B. S. Some critical comments on the methods employed in the expression of leaf saps. Plant Physiol. **4**: 103–112. 1929.
7. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Association of Official Agricultural Chemists, Washington, D. C. 1925.
8. SAYRE, J. D., and MORRIS, V. H. Use of expressed sap in physiologic studies of corn. Plant Physiol. **6**: 139–148. 1931.

NUCLEAR DIVISION IN *TRADESCANTIA VIRGINIANA*

BEATRICE LEHMAN AND F. M. ANDREWS

Introduction

The study of *Tradescantia virginiana* L. as reported in this paper is possible only during its period of anthesis. This lasts, in this region, from about May to October, varying somewhat with the season. The blooming period in this plant may, however, be somewhat extended by employing suitable cultural and cutting methods. Even under the most favorable conditions, the period during which it is possible to study the behavior of the nucleus in the cells of the hairs on the staminal filaments is a comparatively short part of the year.

Tradescantia virginiana has been an object of study for many years. A brief summary of this study has recently been given by KÜSTER (**2**). Although many problems have been solved by work on *Tradescantia virginiana,* still others remain for investigation. Among these may be mentioned: (1) The possible occurrence of intercalary division in the staminal hairs; (2) the position of granules on the protoplasmic strands in the cells of the staminal hairs; (3) chromosome kinetics; (4) BECKER'S (**1**) oxidation processes and nuclear division; (5) the effect of chemicals on nuclear division, etc.; and (6) questions of diapedesis. TELEZYNSKI'S statement that the cells of the staminal hairs remained living for eight days in paraffin oil also certainly merits further investigation.

The following points have been investigated and briefly recorded in this study of nuclear division in *Tradescantia virginiana,* namely, the effects of temperature, carbon dioxide, ether, chloroform, iodine, and fluorescein. In some cases the effects on the protoplasm of *Elodea canadensis* has been mentioned for the sake of comparison.

Experimentation

EFFECTS OF TEMPERATURE ON NUCLEAR DIVISION

The lower temperatures employed in this work were regulated by the use of a MOLISCH freezing box containing dry ice, and the medium and higher temperatures were regulated by using a thermostat, or a heating stage, according to requirements. Table I presents the average results obtained with ten specimens studied for each of the different temperatures recorded. Reading downward the figures in each column of the table indicate the time required for each stage in the nuclear division, and the total time for the nuclear division at the given temperature is recorded at the foot of each column. No divisions occurred at 18° C. The various "stages"

here recorded are the same stages of nuclear division as were figured by STRASBURGER (3).

Table I shows that a temperature of 28° C. was the most favorable, in the stages mentioned, causing nuclear division in 69 minutes, which is 11 minutes less than the total time of the stages recorded by STRASBURGER. Even at 27.5° C. the nucleus divided somewhat more slowly (taking 5 minutes longer, on the average) than indicated by STRASBURGER'S stages. A very interesting variability is shown in the length of time required for completion of nuclear division, beginning with no division at all at 18° C. and rising abruptly to 340 minutes at 19° C. With increasing temperature the time required decreases to 69 minutes at 28° C., which was the optimum average temperature for the specimens studied. Above 28° C. the nuclear divisions proceeded more and more slowly until at 40° C. 330 minutes were required, almost the same period of time as was required at 19° C. Above 40° C. no divisions occurred.

When the temperature is raised or lowered gradually the division of the nucleus is not so noticeably affected as when there is a sudden rise or fall. If the cell is heated to 35° C. suddenly the division is slow or ceases entirely. When the temperature of the oven which was 29° C. was raised in 15 minutes to 38.5° C. nuclear division took place slowly. When heated to 41° C. the nucleus did not divide nor did it recover if it had begun to divide. At a temperature of 18° C. or below, no division occurred, nor was there apparent recovery. It was found that the protoplasm of *Tradescantia virginiana* can remain active at a temperature higher than that which will allow nuclear division. If the temperature has not been too high nor too low, the protoplasm will resume movement after a return to normal conditions.

EFFECTS OF CARBON DIOXIDE

A few observations were made to determine the effects which CO_2 has upon nuclear division of *Tradescantia*. We have measured, first, the length of time required to stop nuclear division; second, the length of time CO_2 can be permitted to act and the nucleus still recover and continue division; and third, the length of time required to kill the nucleus with CO_2.

Carbon dioxide was applied for periods of 20, 10, 5, and 1 minute, and 30, 15, and 10 seconds to different cells whose nuclei were in the process of division. In each case pure moist fresh air at the proper temperature was drawn over the specimens containing the dividing nuclei for 30 to 60 minutes after the application of the CO_2. On the average the nuclear division stopped almost instantly on the application of pure CO_2 or within 2 to 4 seconds, which simply indicates the length of time necessary for the CO_2 to penetrate the cell wall and protoplasm and to reach the nucleus. In treated cells with dividing nuclei, the nuclei failed to recover, except where the CO_2 was applied to the dividing nucleus of the staminal hairs for only 10 sec-

TABLE I

TIME (IN MINUTES) BETWEEN STAGES OF NUCLEAR DIVISION IN RELATION TO TEMPERATURE

STAGES	TEMPERATURE																
	18° C.	19° C.	20° C.	21.5° C.	22.5° C.	24° C.	25.5° C.	26° C.	27.5° C.	28° C.	29° C.	31° C.	32.5° C.	35° C.	37.5° C.	40° C.	41° C.
Stage 3–4	0	40	30	25	20	18	14	12	10	8	10	15	20	25	30	40	0
Stage 4–5	0	40	30	25	20	18	14	12	10	8	10	15	20	25	30	40	0
Stage 5–6	0	20	15	11	13	10	9	7	5	3	5	10	10	15	20	30	0
Stage 6–7	0	20	15	14	13	12	9	7	5	3	5	10	10	15	20	30	0
Stage 7–8	0	20	15	14	13	12	9	7	5	3	5	10	10	15	30	30	0
Stage 8–9	0	40	30	25	20	18	14	12	10	8	15	20	20	25	30	40	0
Stage 9–10	0	80	60	45	40	28	24	22	20	18	25	30	40	45	50	60	0
Stage 10–11	0	80	60	45	40	28	24	22	20	18	25	30	40	45	50	60	0
Total	0	340	255	204	179	144	117	101	85	69	100	140	170	210	260	330	0

onds. In this case, recovery was apparent in 10 minutes after fresh moist air was drawn over the cell. The nucleus was killed by an application of pure CO_2 for 15 seconds.

After an application of CO_2 for 10 minutes, the protoplasm of the young leaf of *Elodea* recovered in 3 minutes; but after an application of CO_2 for 30 minutes there was no recovery of the protoplasm in the cells of the young leaf of this plant, even after fresh moist air was drawn over the specimen for 25 minutes.

Effects of ether

The effect of ether upon the protoplasm and the nuclear division of the staminal hairs of *Tradescantia* and upon the protoplasm of the leaf of *Elodea* was ascertained by the following experiments.

The vapor from a saturated solution of ether in distilled water was drawn over a cell of *Tradescantia virginiana* containing a dividing nucleus; it continued to divide for 5 to 7 minutes and then stopped. When fresh moist air was then drawn over the specimens they recovered in 15 minutes.

After the application of ether vapor for 30 minutes, the nucleus of a cell of *Tradescantia* which had stopped dividing recovered under the influence of fresh moist air in 20 minutes. It is obvious that ether vapor is less destructive to the nuclear division of plants than CO_2.

A cell of *Tradescantia* with a dividing nucleus was placed for 15 minutes over strong ether in a ring cell on a slide; 30 minutes were required for recovery after this treatment, although in some cases even a 5- to 10-minute exposure was sufficient to kill such a dividing cell under these conditions.

Elodea frequently recovered immediately after having been treated with strong ether vapor by the ring-cell method for 15 minutes. On the other hand, as much as 1 hour or more was often required to kill the cell when thus subjected to strong ether vapor.

When the hairs from a staminal filament of *Tradescantia* showing nuclear divisions, and a leaf of *Elodea,* both of which showed active protoplasmic movement at the beginning, were placed side by side in a saturated solution of ether water in amounts sufficient to cover them, the nuclear division in *Tradescantia* was stopped in 30 seconds and the movement of the protoplasm of *Elodea* in 2 minutes. No recovery occurred in either case. Ether is especially harmful to nuclear division and protoplasmic movement when in direct contact with the cells. The vapors, however, are not so harmful, and the cells may even recover some time after its application.

Effects of chloroform

When a current of air was drawn through distilled water saturated with chloroform all nuclear divisions in the staminal hairs of *Tradescantia* were stopped in 60 seconds and there was no recovery. Actively moving protoplasm in the cells of the leaves of *Elodea* was stopped in 5 minutes after

the chloroform vapor was admitted; and even after 15 minutes, the use of fresh moist air failed to bring about its recovery.

One-minute submergence in water saturated with chloroform was sufficient to kill the protoplasm of *Elodea*; and nuclear division in *Tradescantia* was stopped instantly when the cells were submerged in strong chloroform water.

Effects of iodine

The effects of iodine upon the nuclear division of cells of staminal hairs of *Tradescantia* and on the protoplasmic movement of the cells of *Elodea* were investigated.

The vapor from a 5 per cent. solution of iodine was drawn over specimens of *Tradescantia* and *Elodea* for 30 minutes. The vapor had no effect on either, even in 1.5 hours. When, however, specimens of *Tradescantia* and *Elodea* were placed directly in a 5 per cent. solution of iodine, the cells were immediately stained brown, nuclear division and protoplasmic movement stopped at once, and the cells could not be made to recover. Twelve parts of 5 per cent. iodine solution in 500 parts of water stopped division, and the cells were stained deep yellow at once. The same strength of iodine acting on protoplasm of *Elodea* caused rapid decrease in its movement, and after 20 minutes it was completely stopped. No recovery occurred. One cc. of 5 per cent. iodine in 1 cc. of distilled water stopped the protoplasmic movement instantly and no recovery took place.

Specimens of *Tradescantia* with dividing nuclei and leaves of *Elodea* with actively moving protoplasm were placed on a glass ring cell containing a saturated aqueous solution of iodine. This stopped the division and apparently killed the nucleus almost at once. The protoplasm of leaf cells of *Elodea* stopped moving in 5 minutes and did not recover.

Effects of fluorescein

A beautiful green solution of fluorescein was obtained by placing several grains of fluorescein in distilled water. The dividing nuclei of staminal hairs of *Tradescantia,* mounted in this solution, were stained yellow within 5 to 10 minutes. The dye had no effect on the nuclear division and all of the various stages could be readily followed.

Indiana University
Bloomington, Indiana

LITERATURE CITED

1. Becker, W. A. Vitalbeobachtungen über den Einfluss von Methylenblau und Neutralrot auf den Verlauf von Karyo- and Kytokinese. Cytologia **4**: 135–157. 1929–1933.
2. Küster, Ernst. Hundert Jahre Tradescantia. pp. 1–32. 1933.
3. Strasburger, E. Handbook of practical botany. 7th ed. p. 436. Translated by W. Hillhouse.

GOTTLIEB HABERLANDT
1854

BRIEF PAPERS

GOTTLIEB HABERLANDT
1854

(WITH PLATE III AND ONE FIGURE)

On November 28th of this year a great German botanist of the older generation will be eighty years old. The writer of this short biographical sketch was his student and it is with a feeling of sincere admiration that he thinks of the life and work of his great teacher.

GOTTLIEB HABERLANDT was born under the Austro-Hungarian monarchy at Ungarisch-Altenburg, where his father taught classes in natural sciences at the government agricultural school. The father's interest in biology as well as his talent for drawing and music were inherited by his son.

In 1873 HABERLANDT entered the University of Vienna and was much attracted by JULIUS WIESNER'S lectures on the anatomy and physiology of plants. In 1874 his first botanical paper, about cellulose in cork tissues, appeared in the "Oesterreichisch botanische Zeitschrift."

HABERLANDT was not very much influenced by JULIUS WIESNER but he admired him and appreciated his teacher's friendly interest. While a student in Vienna it was the reading of the works of C. NÄGELI, W. HOFMEISTER, HUGO VON MOHL, and FRANZ UNGER, but especially of JULIUS SACHS, through which HABERLANDT trained himself. As a student he published several papers, among which are an investigation of lenticels and a study of the winter coloring in perennial leaves. The last named paper was also accepted as a thesis for the Ph.D. degree which he obtained in 1876. In the autumn of 1877 he went to SIMON SCHWENDENER in Tübingen. HABERLANDT had already conceived the idea of a physiologic interpretation of plant anatomy which SCHWENDENER had suggested in "Das mechanische Prinzip im anatomischen Bau der Monokotylen." In Tübingen HABERLANDT undertook his first independent investigation, "Die Entwicklungsgeschichte des mechanischen Gewebesystems."

SCHWENDENER recognized in HABERLANDT his most talented pupil and later chose him to be his successor in the chair of general botany at Berlin.

HABERLANDT started his research work with a very definite program. He intended to investigate, in the course of years, one system of plant tissues after another and to incorporate finally these investigations into a handbook of physiological plant anatomy. He decided to start with the chlorophyll bearing photosynthetic system, because it was typical for plants and no danger existed here of his being misled by false analogies with animals.

HABERLANDT'S method was to reach conclusions about the relations between structures and functions of tissues on the basis of purely anatomical investigations, and his early deductions were confirmed by later experiments carried out by A. F. W. SCHIMPER.

During the autumn of 1878 HABERLANDT was admitted to a docentship in botany at the University of Vienna. In 1880 he received a call to the Technische Hochschule in Graz, in Austria. There he was made acting professor of botany and simultaneously he became privatdocent at the University.

HABERLANDT was very happy in the beautiful alpine city of Graz with its lovely surroundings and proximity to the Adriatic Sea. In the year following his appointment in Graz, HABERLANDT married CHARLOTTE HAECKER to whom he had become engaged in Tübingen. In 1884 he became professor extraordinarius and in 1888, after LEITGEB'S death, ordinarius at the University.

The first investigation which HABERLANDT undertook in Graz was on the apical growth of the phanerogams. He emphasized the function of the apical cell, which he interpreted through the formation of segments, in contrast to SACHS who defined the apical cell as merely a gap in the constructive system of the cell walls of the vegetative point. After having studied the assimilatory system, HABERLANDT intended to take up the conductive system including the vascular bundles. He took up first the vascular bundles in the petioles of ferns and second the latex tubes which he explained as having the function of typical conductors for the assimilation products of plants.

Meanwhile SCHWENDENER had been called to Berlin and had founded his Botanisches Institut. There his pupils worked out problems in the field of physiological plant anatomy. These papers appeared without a very definite program and HABERLANDT realized that a fundamental classification of tissue systems from the viewpoint of physiological plant anatomy was urgently needed. He decided to write his handbook without waiting until all the necessary Vorarbeiten should be completed. In 1884 appeared the first edition of the "Physiologische Pflanzenanatomie."

The "Physiological Plant Anatomy" saw six editions and is still much read while contemporary text-books of plant physiology are no longer in use. In his charming memoirs, "Erinnerungen, Bekenntnisse und Betrachtungen" (Berlin, Julius Springer, 1933), which every botanist should read, HABERLANDT expressed the hope that some day somebody else might give an independent treatment of evolutionary physiological anatomy of plants (Entwicklungphysiologische Anatomie) as a counterpart of the functionally physiological anatomy which he formulated in his handbook.

HABERLANDT'S "Physiologische Pflanzenanatomie" found many readers throughout central, northern, and eastern Europe and in Japan. It was translated into English but never became very popular in France.

It was in the winter semester, 1893–94, that the author of this biographical sketch first attended HABERLANDT'S lectures on physiological plant anatomy at the University of Graz. I have a very vivid recollection of my teacher. His lectures were extremely inspiring and beautifully organized. A natural talent for drawing, as well as the magnificent anatomical and flower models prepared by his technician, H. GASSER, added to their instructiveness. In the microscope room he visited every advanced student several times a day, giving advice, suggesting new viewpoints, explaining new ideas. He had eminently what the Germans call a philosophical mind, never being satisfied with the establishment of mere facts, but always attempting a theoretical interpretation.

As professor ordinarius of botany and director of the botanical institute and garden, HABERLANDT enjoyed a great deal of independence in administering his small domain. In matters of budget he dealt directly with a department head in the provincial governor's office who in turn merely represented the imperial and royal secretary of education. HABERLANDT was twice elected dean of the faculty of philosophy in the University but declined election to the office of rector magnificus.

The other professor of botany, CONSTANTIN VON ETTINGSHAUSEN, whose assistant I was, did not always cultivate the best of personal relations with HABERLANDT. My position was therefore a very delicate one but HABERLANDT always treated me in a very friendly and considerate manner. I am proud to be one of his Schüler. During his 44 years of active teaching in Vienna, Graz, and Berlin, HABERLANDT must have had hundreds of advanced students. Only six of them, including the writer of this paper, reached university positions, and of these six two are dead, EDUARD PALLA and CARL ERICH CORRENS.

At this time fell also a visit to the botanical garden of Buitenzorg in Java (1891–92). Fruits of this trip were studies of the transpiration of plant leaves and a charming volume "Eine botanische Tropenreise," which had three editions and is still a very popular book among botanists, owing to HABERLANDT'S broad scientific observations and artistic presentation and to his masterful pencil sketches which accompany the text.

In 1909 SCHWENDENER retired, at eighty years of age, from the chair of plant physiology at the University of Berlin and he suggested that HABERLANDT be his successor. The latter began his lectures in Berlin in the autumn of 1910.

With a heavy heart HABERLANDT left his beautiful Botanisches Institut in Graz and the lovely alpine city with his many congenial friends. He

had not accepted the call to Berlin until he had been promised a new institute for plant physiology with greenhouses and gardens, but it was three years before HABERLANDT could move his institute to Dahlem where he also had his official residence.

HABERLANDT worked at that time with cell division hormones, wound hormones, necro-hormones, lepto-hormones, etc. He also started a serial publication for his own and his students' contributions called "Beiträge zur allgemeinen Botanik." The Beiträge did not continue after the second volume owing to the unfavorable financial conditions in Germany. HABERLANDT gave normally during autumn and winter at Dahlem a course in anatomy and physiology of plants. This was changed later to physiological plant anatomy, when the professor extraordinarius of general botany was giving a lecture course on plant physiology.

During the summer semester HABERLANDT gave what he called "Grundzüge der Botanik." This was a survey course touching, in the short span of three months, four times a week, on cytology, anatomy, organography, physiology, and the taxonomy of thallophytes, bryophytes, pteridophytes, and spermatophytes. This course was held at the University building in the city of Berlin.

HABERLANDT would have liked to add to his "Physiologische Pflanzenanatomie" chapters on the anatomy of reproductive organs as well as on the evolutionary physiology of tissue systems. Another wish was to write a text-book of botany for artists and craftsmen. His own paintings in water colors and oil had splendidly prepared him for such a task. He was rarely qualified to do it. But soon after he had organized his new Pflanzenphysiologisches Institut in Dahlem the world war broke out. His four sons served in it, two in the German and two in the Austrian army. All returned unharmed. HABERLANDT's first wife had died in February of 1910 and he married again in August of 1914. He had seven children, five by his first wife and two were born during the war from his second marriage.

During the world war HABERLANDT served on various food boards and after peace had returned he continued his botanical researches, producing valuable papers to date. In a recent letter he assured the writer of this biography that his eyes are still as serviceable for microscopic work as they were thirty years ago. May he be spared for a long time, for the benefit of botany, and reach the nineties as did his great teacher, SCHWENDENER.

HABERLANDT is one of a great group of German botanists. Of the others, NÄGELI, SACHS, DE BARY, STRASBURGER, SCHWENDENER, ENGLER, CORRENS, PFEFFER, WIESNER, KERNER, and WETTSTEIN have all passed away. HABERLANDT and the 78-year old HANS MOLISCH are the only survivors of that group of brilliant botanists whose race was German and who belonged to Germany, Austria, and Switzerland. His is a good illustration of the best

type of German university professor; a thorough scientist, an excellent teacher, a man of broad scientific and humanistic interests, upright and independent in his convictions. The old German universities had many men of this type and it is to be hoped that they also may be found in the new generation.

Perhaps it was the humanistic education that created the intellectual leaders among the scientists of HABERLANDT'S generation. The gradual disappearance of this background may account for the smaller number of brilliant men of scientific thought among the much larger masses of scientifically trained men in our time. The greatest change in the history of civilization is the disappearance of classical education. It has ruled the intellectual life of European mankind from the time of the Renaissance to the world war. HABERLANDT still belongs to the intellectual aristocracy of the classical era.—A. C. NOÉ, *University of Chicago.*

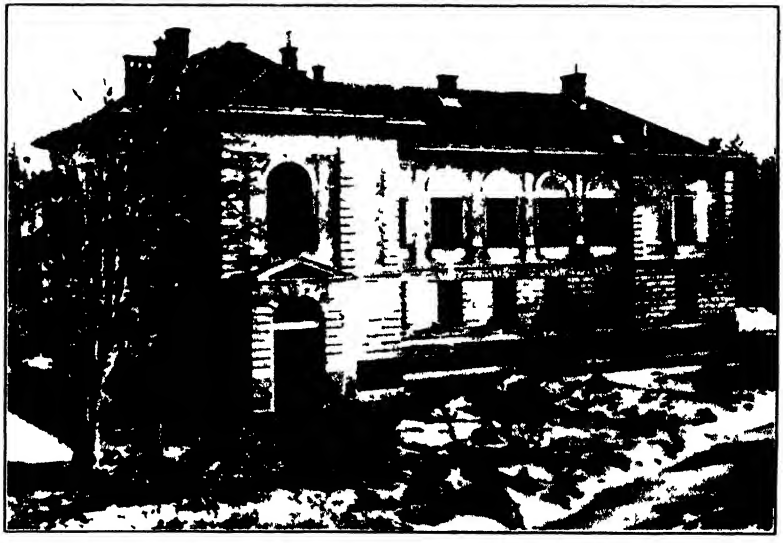


FIG. 1. Institute for plant physiology and anatomy, University of Graz, Austria.

DEMONSTRATION OF SOME OF THE MECHANISMS INVOLVED IN THE ASCENT OF SAP IN PLANTS

(WITH ONE FIGURE)

The usual demonstration of osmosis with sugar solution separated from water by a differentially permeable membrane is unsatisfactory as a model representing the plant mechanisms involved in sap ascent. Beginning students are often confused because in the demonstration they see a solution rising in a tube due to a force exerted not from above, as it is in the plant, but from below. A simple rearrangement of the materials ordinarily used provides a very convincing demonstration of the process of osmosis, and at the same time shows more exactly how osmosis in the leaf cell brings about the rise of water in the stem below. The addition of a capillary evaporating surface to the apparatus then makes the picture of the mechanisms involved and their coordination quite complete.

Figure 1 (A) shows how the apparatus is arranged. A glass tube (t), one or more meters long, communicating above with a short glass cylinder

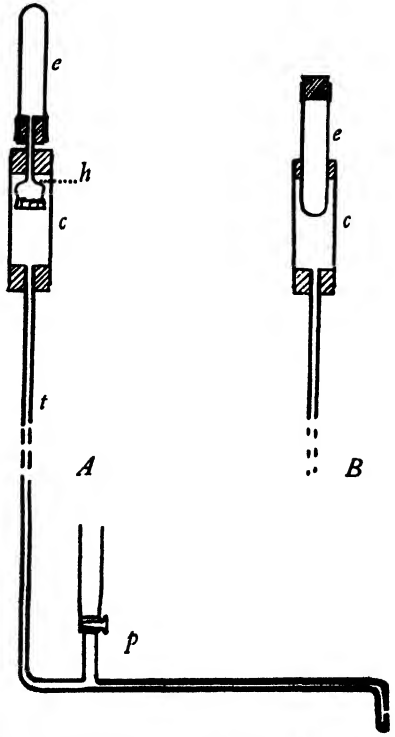


FIG. 1. Apparatus for demonstration of some of the mechanisms involved in sap ascent.

(dimensions about 5×20 cm.) is fastened upright, the lower end stoppered, and the whole filled with water, excluding all air bubbles. A membrane is tied over the bulb end of a short thistle tube (h), the tube filled with water, and sealed into the cylinder as shown. A small lump of copper sulphate in the water stains the membrane blue, and may protect it from decay. Before adding sugar the stopper below is removed. Water filters very slowly through the membrane, and the column of water in the tube (t) falls. Movement of the water may be measured conveniently by means of a potometer (p) attached below. Sucrose crystals are now dropped into the thistle tube from above (through a water-filled funnel attached to the stem of the thistle tube) with the result that the water immediately reverses its direction of flow. This rise of water, following as a result of merely adding a solute, affords an especially convincing demonstration of the power of osmosis.

If, now, a porous clay evaporating cylinder (e) be filled with water and fastened to the end of the thistle tube as shown in the figure, the rise of the water in the tube *as in the plant* will clearly be affected by surface and evaporational forces, as well as by osmosis. Subjecting the apparatus to hot, dry moving air will cause the water to move rapidly in the tube. Surrounding the evaporating surface with a humid atmosphere, on the contrary, will cause osmosis to work alone, and the solution will be forced out through the porous clay walls, superficially simulating guttation. When osmosis dominates over evaporation, the membrane is expanded to its maximum (as would be a leaf-cell wall and protoplast under these conditions) and bulges downward. When the rate of evaporation is very high, the membrane becomes slack or may even bulge upward. It is a good exercise for the student to explain the various observed rates of water movement in terms of the relative vapor tension in the water, solution, capillaries, and atmosphere, and clarifying to see the working together of the different processes involved in this simple model.

The tabulation (p. 858) gives data representative of the type of results that may be expected.

For more advanced students acquainted with the use of precipitated membranes in osmometers, a modification of this apparatus may be used. Here the porous cylinder alone serves as both the osmotic and the evaporating unit, thus approaching a step nearer the situation in the leaf cell where there may be no essential difference except in position between the absorbing and evaporating parts. Copper ferrocyanide is precipitated into the walls of the cylinder according to the usual procedure. After being filled with syrup or sugar solution, the porous cylinder is tightly stoppered and fitted into the water cylinder (c), so that only its lower half is immersed, the upper part being exposed to evaporation (fig. 1 B). A narrow rub-

Conditions	Rate of rise per minute	Observed rate of movement in capillary tube of potometer per minute
	mm.[3]	*cm.*
Before adding evaporating surface—osmosis alone	19.9	7.6
Evaporation plus osmosis. Quiet air	26.2	10.0
Evaporation plus osmosis. Hot, moving air	52.3	20.0
Osmosis. Evaporation prevented by moist towel and beaker	14.6	5.6

Membrane: pig bladder
Room temperature: 26°C.
Weight of sucrose: 50 gm.
Volume thistle tube: 32 ml.
Volume evaporating cylinder: 50 ml.
Total height of water column: 1.5 m.

ber ring fitted around the porous cylinder makes the seal between it and the water cylinder. This apparatus works as does the one previously described, except, of course, that it will not "guttate" unless the membrane is incomplete at the stoppered end.—Ernest H. Runyon, *92 Edgewood Ave., La Grange, Illinois.*

A NOTE ON FRUITING APPLE SPUR PHYLLOTAXY

The writer was considerably interested in Hubbell's paper[1] in the April, 1934, issue of Plant Physiology on blind wood in roses, particularly in his observation that the fruitful shoots of the hybrid tea rose, Mme. Butterfly, averaged 7.3 nodes per shoot, while the unfruitful shoots averaged 4.9 nodes per shoot. In 1927 while at Oregon State College a nearly identical observation was recorded with respect to apple cluster bases. Usually the fruiting spurs showed 8 or 10 leaves below the terminal fruit stalks on the cluster base; 8 leaves were present in a phyllotaxy of $\frac{3}{8}$, or 10 leaves in a phyllotaxy of $\frac{4}{10}$. Spurs unfolding small numbers of leaves were seldom fruiting. The rule is not invariable, but the majority of cases adhere closely to it. Spurs of the Starking apple in the University of Wisconsin orchards this spring showed the arrangement beautifully. Incidentally, 8 nodes or leaflets on shoots of hybrid tea roses usually represent one complete phyllotaxy in $\frac{3}{8}$ arrangement. In the apple in strong fruiting spurs, *one complete*

[1] Hubbell, D. S. Causes of blind wood in roses. Plant Physiol. 9: 261–283. 1934.

phyllotaxy of leaves is usually laid down on the cluster base below the terminal flower embryos. In cases where more than 8 leaves appear on the spur, the internode length[2] beyond the eighth node increases very rapidly, and fruiting decreases abruptly.—HAROLD L. COLBY, *University of Wisconsin, Madison, Wisconsin.*

[2] ROBERTS, R. H. Off year bearing and apple spur growth. Jour. Pomol. and Hort. Sci. 2: 16–37. 1920.

NOTES

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists will be held at Pittsburgh, December 27–29, 1934. The William Penn Hotel has been selected as headquarters for the Society. Early reservations should be made, as the demand for accommodations may be heavy.

At this time, early in October, only a few facts about the meetings can be presented. A joint symposium on plant hormones is scheduled with the Botanical Society of America for Thursday afternoon; a joint session with the American Society for Horticultural Science has been arranged for Friday morning; and a joint session with section G, A. A. A. S., for Friday afternoon. There will probably be three other sessions for the reading of papers, the usual business meetings, and the annual dinner. Tickets for the dinner should be obtained promptly on arrival in order that proper arrangements may be made to accommodate everyone.

Life Membership Committee.—The committee whose duty it is to make the award of the CHARLES REID BARNES life membership in the American Society of Plant Physiologists has been appointed by President BURTON E. LIVINGSTON, who was the first CHARLES REID BARNES life member in 1926. The members of the committee are Dr. H. A. SPOEHR, of the Carnegie Institution of Washington, chairman, Dr. JAMES B. OVERTON, University of Wisconsin, and Dr. T. G. PHILLIPS, University of New Hampshire. The by-laws provide that the award is to be made to a plant physiologist from foreign lands every fifth year. The first such award may be made this year. The announcement of the award is made at the annual dinner.

Council Representation, A. A. A. S.—The two representatives of the American Society of Plant Physiologists in the Council of the A. A. A. S. appointed by President LIVINGSTON are Dr. CHARLES O. APPLEMAN, University of Maryland, and Dr. CHARLES A. SHULL, University of Chicago.

Exhibition of A. A. A. S.—The American Association's Exhibits Committee (Dr. F. C. BROWN, chairman) states that the annual science exhibition will this year be larger, better, and more comprehensive than heretofore. It will include many exhibits by research workers, as well as numerous exhibits of apparatus by commercial firms. Individuals and institutions are invited to correspond with Dr. BROWN concerning the exhibition of new research apparatus and techniques, preferably arrangements that show motion or visible changes, or which are otherwise generally

attractive. This is an opportunity for plant physiologists to give valuable service to their science, and to science in general.

Sixth International Botanical Congress.—The plant physiology section of the Sixth International Botanical Congress at Amsterdam will consider six great topics: Photosynthesis, phyto-hormones, oxidation-reduction and metabolism, permeability and accumulation of mineral elements, translocation of plastic materials, and influencing the cycle of development in plants. There will be a general paper on phyto-hormones, accompanied by several special papers. In connection with the translocation of plastic materials, the problem of the submicroscopic structure of cell walls will be considered. These topics indicate the broad fundamental character of the proposed sessions. All botanists who can do so should journey to Amsterdam in September, 1935, to share in the advantages and privileges of international cooperation in scientific progress.

Carotenoids.—Volume 31 of the Monographien aus dem Gesamtgebeit der Physiologie der Pflanzen und der Tiere is an interesting and valuable summary of our knowledge of the carotenoid pigments. The author is Dr. L. ZECHMEISTER, director of the Chemical Institute of the University of Pécs, Hungary. The major part of the work is devoted to the plant carotenoids; the chapter on animal carotenoids is very brief. It covers the historical development of research in this field, the chemical nature, physiology, and rôle of the pigments, their relationship to other chemical entities, methods of investigation of constitution, isolation, and quantitative estimation. A special section gives detailed information about each of the twenty pigments which have been found in plants. Most of these are polyene compounds containing oxygen. Two are hydrocarbons (carotene and lycopene), and all but a few belong in the C_{40} series of compounds. The work reflects and emphasizes the rapid progress made in the last five years in unraveling the different constitutional problems which the carotenoids present. The book is published by Julius Springer, Berlin, at RM 28 for brochure style, and RM 29.40 for cloth-bound copies.

Respiration.—The appearance of a posthumous booklet on Cellular Respiration by NORMAN U. MELDRUM recalls also the brief monograph on Respiration in Plants by W. STILES and W. LEACH two years ago. MELDRUM's monograph, 116 pages with glossary, appendix, and index, contains seven chapters: Introduction; dehydrogenase systems; the work of WARBURG; oxidases, peroxidases, and catalase; the cytochrome system; the glutathione system; and modern developments. The author purposely omits the oxidation-reduction potential problems as too controversial, inaccurate, and too difficult to interpret.

The STILES and LEACH monograph is of almost the same size, 124 pages. There is an introductory chapter, and chapters on the respiration of normal plants under aerobic conditions, anaerobic respiration, and the mechanism of respiration.

The MELDRUM monograph may be obtained from Methuen & Co. Ltd., 36 Essex St. W. C., London, at 3*s.* 6*d.*, postage extra. The other is published by the Dial Press, 152 West 13th St., New York, at $1.50 per copy. Both are useful little books which should be in the hands of plant physiologists.

Plant Biochemistry.—An Introduction to Plant Biochemistry by CATHERINE CASSELS STEELE, is published by G. Bell & Sons Ltd., London. There are 27 chapters, which fall into seven parts. The general ground covered is indicated by the titles of these larger divisions: Introduction; alcohols, fatty acids, fats and oils; aldehydes, ketones, and carbohydrates; plant acids; proteins and related compounds; cyclic compounds; and plant metabolism. The earlier sections deal with the nature of the constituents rather than with their biochemical origin. A large amount of information has been brought together, and the book will no doubt find many interested readers. The quoted price is 15 shillings, net.

Reshaping Agriculture.—This little book by Dr. O. W. WILLCOX looks forward to the time when agriculture will be so efficient that 80 per cent. of our rural population will not be needed as tillers of the soil. He believes that we now have enough technical knowledge about plants that if it were all utilized in practice there would be but one limiting factor on yield,—the "quantity of life," or innate capacity for growth belonging to the species. He goes to the opposite extreme of the old Malthusian doctrine of ultimate starvation from inadequate food supplies to a plethora of abundance that needs to be "managed," socially controlled. Chapter VI deals with the abolishing of weather hazards in farming, a subject that ought to interest the present administration in Washington. The chapter on social intervention in agriculture should be read by everyone interested in the real welfare of mankind; for it is only by knowing what "new dealers" are thinking that defense mechanisms against foolishness can be constructed. Dr. WILLCOX seems privately to feel that botanists and ordinary agriculturists have done scant courtesy to his ideas concerning agrobiology. As this little volume, Reshaping Agriculture, published by W. W. Norton & Co., New York, costs only $2.00, it would at least be easily possible to follow the author's excursion into the "era of abundance" and its "implementation." The reshaping of agriculture probably has about as good a chance to succeed as the reshaping of business, or the reshaping of human

nature. The reviewer feels that this agricultural social-economic millennium is about as far away as the millennium of the Apocalypse.

Horticultural Plant Breeding.—This volume, Grundriss der gärtnerischen Pflanzenzüchtung, is a general work on applied genetics. The author is H. KAPPERT, of the Landwirtschaftliche Hochschule, Berlin. The sixteen sections cover the range of Mendelian behavior, mutation phenomena, chimaeras, graft hybrids, selection, pure lines, inbreeding, heterosis, etc. The Mendelian behavior is considered in detail, although the entire work is condensed into small compass, 148 pages. Horticulturists will find it a useful work on breeding. It comes in stiff brochure binding at RM 6.80 per copy. The publisher is Paul Parey, Berlin.

Pathology of Mitosis.—A monograph entitled Pathologie der Mitose has been prepared by Dr. GEORG POLLITZER, of the University of Vienna. The discussion deals with the abnormalities of mitosis, mainly of animal cells, although a number of cases are described from plant materials. The author first considers the morphology of abnormal karyokinesis, then disturbances in the cell division rhythm, the comparative etiology of abnormal mitoses, actinic effects, chemical effects, and the effects of electric currents. A brief chapter at the close considers the problem of specificity, that is, specific cell reactions to disturbing factors. The work is published by Gebrüder Borntraeger, Berlin, at RM 16.20 bound in cloth.

Mammalian Red Cells.—Although not in the domain of plant physiology, attention is called to the publication of volume 6 of the Protoplasma Monographien which are published by Gebrüder Borntraeger, Berlin. The author is Dr. ERIC PONDER, New York University, and the complete title of the book is The Mammalian Red Cell and the Properties of Haemolytic Systems. The earlier chapters discuss methods of counting corpuscles, their dimensions, shape and structure, chemical composition and metabolism, permeability and the phenomenon of osmotic haemolysis. Later chapters consider the properties of haemolytic systems, inhibition and acceleration of haemolysis, resistance series, systems containing sensitizing agents, and miscellaneous forms of haemolysis. The price is RM 22.5 per copy, with cloth binding.

GENERAL INDEX[1]

A

A.A.A.S., Council representation, 861
exhibition, 861
ABRAMSON, H. A., Electrokinetic phenomena, 698
Absorption and root respiration, relation between, 283
of ash constituents, effects of potassium, 399
of nitrate by the young wheat plant, influence of shorter light rays, 127
spectra of alpha and beta carotenes and leaf xanthophyll at room and liquid nitrogen temperatures, 179
spectra, quantitative, of common carotenoids, 693
Accumulation of petroleum spray oils in leaves, twigs, and fruit of citrus trees, 699
Acetaldehyde, production of by tomatoes, 359
Acidity, hydration of solute ions in relation to, 107
Acids, amino, relation to carbon dioxide and mycelium production of *Fusarium oxysporum*, 823
Activity, oxidase, effect of high frequency sound waves, 385
Advances, recent, in plant physiology, 397
Aerated and non-aerated culture solutions, anatomical and histological differences between barley roots grown in, 389
Aeration, 521
physiological aspects of effect of continuous soil on plant growth, 567
Agriculture, reshaping, 863
Alcohol, production of by tomatoes, 359
Alkalinity, hydration of solute ions in relation to, 107
Alpha carotene, absorption spectra, 179
Amendment of constitution, 184
Amino forms of nitrogen, sugars, and enzyme activity of sugar cane, effects of potassium, 453
Ammonium assimilation in tomato and apple, 31
nutrition, metabolism of etiolated seedlings as affected by, 351
Amounts of bound and free water in an organic colloid at different degrees of hydration, 143
Amylase studies, 463
Analyses, application of quantitative spectral to binary mixtures of the common carotenoids, 681
Anatomical differences between roots of barley grown in aerated and non-aerated culture solutions, 389
ANDERSON, A. K., Relation of amino acids to carbon dioxide and mycelium production of *Fusarium oxysporum*, 823
ANDREWS, F. M., Nuclear division in *Tradescantia virginiana*, 845
Annual election, 695
Apparatus, 129, 174, 786, 790, 856
bomb for calorimeter, 325
calorimeter, 324
for root respiration, 284
Apple, assimilation of ammonium and nitrate nitrogen, 31
effects of humidity on metabolism, 217
spur phyllotaxy, fruiting, 858
Apples, effect of ethylene, ethylene chlorohydrin, and ultra-violet light on carbohydrate content of stored, 637
Application of calorimetric methods to ecological research, 323
of sodium nitrate, effects on flower and blind wood production, 270
Ascent of sap in plants, mechanisms involved, 856
Ash constituents, effects of potassium upon absorption and migration, 399
Assimilation, nitrogen, and effects of soil pH in tomato, 52
of ammonium and nitrate nitrogen, 31
Award, life membership, 183

B

BALDWIN, H. I., Germination of the red spruce, 491
Barley roots grown in aerated and non-aerated culture solutions, anatomi-

[1] Papers published in Plant Physiology are indexed in *The Agricultural Index*, the H. W. Wilson Co., New York City.

cal and histological differences between, 389
BARNES, CHARLES REID, Botanical Laboratory, 697
BARTON-WRIGHT, E. C., Recent advances in plant physiology, 397
Base element relationships of peas grown in water culture under various light exposures, 301
BEALL, RUTH, The soil as direct source of carbon dioxide for ordinary plants, 237
Beans, soy, 60
soy, relations of germination to temperature and length of incubation time, 1
BENEDICT, H. M., Effect of soft x-rays on germination of wheat seeds, 173
Benzoic acid content of cranberries, relation to keeping quality, 631
Berkeley meeting, 695
Beta carotenes, absorption spectra, 179
Binary mixtures of common carotenoids, application of quantitative spectral analyses, 681
Biochemistry, plant, CATHERINE CASSELS STEELE, 863
plant, W. E. TOTTINGHAM, 698
Blight, pahala, 427
Blind wood in roses, causes, 261
Bluegrass, Kentucky, responses to variations in temperature, light, cutting, and fertilizing, 83
Boston meeting, 183
Botanical Congress, Sixth International, 185, 862
laboratory, CHARLES REID BARNES, 697
Botany, progress of, FRITZ VON WETTSTEIN, 398
Bound and free water in an organic colloid at different degrees of hydration, 143
Breeding, horticultural plant, 864
British economic grasses, SIDNEY BURR and DOROTHY M. TURNER, 186
BRYANT, A. E., Comparison of anatomical and histological differences between roots of barley grown in aerated and non-aerated culture solutions, 389
Budding experiments, 269
Buffer, transpiration as a temperature, 167
BULLER, A. H. R., Researches on fungi, 398
BURK, DEAN, Preparation of humate iron and other humate metals, 663
BURKHART, LELAND, Metabolism of etiolated seedlings as affected by ammonium nutrition, 351
BURNS, G. R., Long and short wavelength limits of photosynthesis, 645
BURR, SIDNEY, British economic grasses, 186

C

Calorimetric methods, application to ecological research, 323
Cane, sugar, effects of potassium upon amounts of protein and amino forms of nitrogen, sugars, and enzyme activity, 453
sugar, effects of potassium upon growth, 399
Carbohydrate, and nitrogen relations in wheat plants with reference to type of growth under different environmental conditions, 533
content of stored apples, effect of ethylene, ethylene chlorohydrin, and ultra-violet light, 637
metabolism, 474
-nitrogen and base element relationships of peas grown in water culture under various light exposures, 301
nitrogen ratios with respect to sexual expression of hemp, 731
Carbon dioxide, content of the gas from pea pods, 339
dioxide effects, 846
dioxide, relation of amino acids to, and mycelium production of *Fusarium oxysporum*, 823
dioxide, soil as direct source of for ordinary plants, 237
Carotenes, alpha and beta, absorption spectra, 179
Carotenoids, 862
common, application of quantitative spectral analyses to binary mixtures, 681

for research, 697
quantitative absorption spectra, 693
Carrots, chemical changes in during growth, 671
Cells, mammalian red, 864
Cellular respiration, 862
Chamber, climatic, 129
Chemical methods, 696
methods committee, 397
Chemist, organic, what he can do for plant physiology, 685
Chlorococcum, pure culture grown in darkness, and production of vitamins, 807
Chloroform effects, 848
Chlorophyll, for research, 697
production under various environmental conditions, 59
CHRISTENSEN, R. J., Effect of high frequency sound waves on oxidase activity, 385
CHRYSLER, HELEN L., Amounts of bound and free water in an organic colloid at different degrees of hydration, 143
Citrus trees, penetration and accumulation of petroleum spray oils in leaves, twigs, and fruit, 699
CLAGUE, J. A., Relation of benzoic acid content and other constituents of cranberries to keeping quality, 631
CLARK, H. E., Preparation of aqueous extracts of soluble nitrogen from plant tissues, 817
CLEMENTS, F. E., Effect of soil temperature on transpiration in *Helianthus annuus*, 619
Factors in elongation and expansion under reduced light intensity, 767
CLEMENTS, H. F., Significance of transpiration, 165
Climatic chamber, 129
Clover seedlings, sweet, 43
Cohesion theory, and evaporation in its thermodynamic relation, 653
theory of transpiration, 783
COLBY, H. L., A note on fruiting apple spur phyllotaxy, 858
Colloidal and non-colloidal nitrogen, differences, 275
Colloid, bound and free water in an organic, 143
Committee, chemical methods, 397
life membership, 861
program, 396
STEPHEN HALES prize, 397
Comparison, of anatomical and histological differences between roots of barley grown in aerated and non-aerated culture solutions, 389
of heating and freezing methods of killing plant material for cryoscopic determinations, 369
Composition, gradient, of rose shoots from tip to base, 157
Concentration, hydrogen-ion, of culture solutions, 204
Conducting system, movement of spray oil, 710
Congress, Sixth International Botanical, 185, 862
Constitution, amendment, 184
plant, F MERKENSCHLAGER and M. KLINKOWSKI, 186
Content of the gas from pea pods, carbon dioxide, 339
Corn, losses to the crop caused by leaf injury, 749
Cotton plants, 43
Council representation, A.A.A.S., 861
Cranberries, relations of benzoic acid content and other constituents to keeping quality, 631
Cresoap emulsions of petroleum oils, freezing phenomena, 795
Cryoscopic determinations, comparison of heating and freezing methods of killing plant material, 369
Cucurbita pepo, 353
Culture, pure, of *Chlorococcum* grown in darkness and production of vitamins, 807
solutions, barley roots grown in aerated and non-aerated, 389
water, carbohydrate-nitrogen and base element relationships of peas grown in, 301
Cutting, responses of Kentucky bluegrass to variations in, 83

D

Darkness, pure culture of *Chlorococcum* grown in, and production of vitamins, 807

DAVIDSON, O. W., Preparation of aqueous extracts of soluble nitrogen from plant tissues, 817
DAVIS, ELIZABETH E., Influence of thyroxin on growth of plants, 377
Deficiency, symptoms of potassium, 399
Degrees of hydration, bound and free water in an organic colloid at different, 143
Determinations, comparison of heating and freezing methods of killing plant material for cryoscopic, 369
DEXTER, S. T., Respiratory rate and enzyme activity as related to hardened condition of plants, 831
Salt concentration and reversibility of ice-formation as related to hardiness of winter wheat, 601
Diastase, 175
DICKSON, A. D., Carbohydrate and nitrogen relations in wheat plants with reference to type of growth under different environmental conditions, 533
Differences, anatomical and histological, between roots of barley grown in aerated and non-aerated culture solutions, 389
Dioxide, carbon, content of the gas from pea pods, 339
Directory, 696
Distribution of inorganic solutes, 168
Diurnal changes in carbon dioxide content of pea pods, 342
Division, nuclear, in *Tradescantia virginiana*, 845
DONEEN, L. D., Methods for the preparation of green plant material for the extraction of juices, 839
Drying oven with constant uniform temperature and forced ventilation, 689
DUNGAN, G. H., Losses to the corn crop caused by leaf injury, 749
DUSTMAN, R. B., Effect of ethylene, ethylene chlorohydrin, and ultra-violet light on carbohydrate content of stored apples, 637

E

Ecological research, application of calorimetric methods to, 323
Economic grasses, British, SIDNEY BURR and DOROTHY [illegible] TURNER, 186
EDWARDS, T. I., Relations of germinating soy beans to temperature and length of incubation time, 1
Effect of high frequency sound waves on oxidase activity, 385
Effects of pH, elaboration of nitrogen fraction on, 51
Election, annual, 695
Electrodialysis, extraction of plant tissue by, 33
Electrokinetic phenomena, H. A. ABRAMSON, 698
Element, base, relationships of peas grown in water culture under various light exposures, 301
Elongation under reduced light intensity, 767
EMMART, KATHRYN, Relation of certain amino acids to carbon dioxide and mycelium production of *Fusarium oxysporum*, 823
Emulsions, cresoap, of petroleum oils, freezing phenomena, 795
Endowments, 696
England, New, section, 184
Environment and chlorophyll production, 59
Environmental conditions, carbohydrate and nitrogen relations in wheat plants, 533
Enzyme activity as related to hardened condition of plants, 831
activity, effects of potassium upon amounts of protein and amino forms, 453
Ereptase studies, 468
Errata, v
Ether effects, 848
Ethylene chlorohydrin, effect on carbohydrate content of stored apples, 637
Etiolated seedlings, metabolism of as affected by ammonium nutrition, 351
Evaporation in its thermodynamic relation to cohesion theory and to imbibitional and osmotic pressures of cell constituents, 653
Excised tomato root tips, growth in liquid medium, 585

Expansion under reduced light intensity, 767
Exposure, light, carbohydrate-nitrogen and base element relationships of peas grown in water culture under various, 301
Extraction, of chlorophyll, 61
of juices, method for preparation of green plant material for, 839
of plant tissues by chemical methods, 33
Extracts of soluble nitrogen from plant tissues, preparation of aqueous, 817
EZEKIEL, W. N., Nutritional requirements of the root rot fungus, *Phymatotrichum omnivorum*, 187

F

Factors, limiting, 136
FELLERS, C. R., Relation of benzoic acid content and other constituents of cranberries to keeping quality, 631
Fertilizing, responses of Kentucky bluegrass to variations in, 83
FLINT, L. H., Hydration of solute ions in relation to acidity, alkalinity, and pH, 107
Fluorescein effects, 849
Forced ventilation, a drying oven with, 689
Free water in an organic colloid at different degrees of hydration, 143
Freezing, and heating methods of killing plant material for cryoscopic determinations, 369
influence on carbon dioxide content of pea pods, 346
phenomena in cresoap emulsions of petroleum oils, 795
Frequency sound waves, high, effect on oxidase activity, 385
Fruiting apple spur phyllotaxy, 858
Fruit of citrus trees, penetration and accumulation of petroleum sprays oils, 699
FUDGE, J. F., Nutritional requirements of the root rot fungus, *Phymatotrichum omnivorum*, 187
Fungi, researches on, A. H. R. BULLER, 398
Fungus, nutritional requirements of the root rot, 187
Fusarium oxysporum, relation of amino acids to mycelium production, 823

G

Gas from pea pods, carbon dioxide content, 339
Germinating soy beans, relation to temperature and length of incubation time, 1
Germination, of red spruce, 491
of wheat seeds, effect of soft x-rays, 173
Ginkgo biloba, lateral water transfer, 387
Gradient composition of rose shoots from tip to base, 157
Grafting experiments, 267
Grasses, British economic, SIDNEY BURR and DOROTHY M. TURNER, 186
GREEN, E. L., Gradient composition of rose shoots from tip to base, 157
Growth, associated with changes in composition of gas of pea pods, 342
chemical changes in carrots during, 671
habits of rose shoots, 263
of excised tomato root tips in liquid medium, 585
of plants, influence of thyroxin on, 377
of sugar cane, effects of potassium upon, 399
of wheat under different environmental conditions, 533
physiological aspects of effect of continuous soil aeration, 567
-promotion substances, possible accessory, 207
transpiration and speed of, 168
GUNDERSON, M. F., Production of vitamins by a pure culture of *Chlorococcum* grown in darkness on a synthetic medium, 807
GUSTAFSON, F. G., Production of alcohol and acetaldehyde by tomatoes, 359

H

HABERLANDT, GOTTLIEB, 851
Habits, growth, of rose shoots, 263

Hail, injury, 749
HALES, prize committee, STEPHEN, 397
Hardened condition of plants as related to respiratory rate and enzyme activity, 831
Hardiness of winter wheat and relation of salt concentration and reversibility of ice-formation, 601
HARRISON, C. M., Responses of Kentucky bluegrass to variations in temperature, light, cutting, and fertilizing, 83
HARTT, CONSTANCE E., Some effects of potassium upon growth of sugar cane and upon absorption and migration of ash constituents, 399
Some effects of potassium upon amounts of protein and amino forms of nitrogen, sugars, and enzyme activity of sugar cane, 453
Heating and freezing methods of killing plant material for cryoscopic determinations, 369
Helianthus annuus, effect of soil temperature on transpiration, 619
Hemp, sexual expression of with respect to carbohydrate-nitrogen ratios, 731
HENDERSON, LETA, Relation between root respiration and absorption, 283
High frequency sound waves, effect on oxidase activity, 385
H ion of nutrient solution, 45
Histological differences between roots of barley grown in aerated and non-aerated culture solutions, 389
HOMAN, CLYDE, Evaporation in its thermodynamic relation to the cohesion theory and to imbibitional and osmotic pressures of cell constituents, 653
HOOVER, S. R., Preparation of humate iron and other humate metals, 663
HORNER, C. K., Preparation of humate iron and other humate metals, 663
HUBBELL, D. S., Causes of blind wood in roses, 261
Humate iron, preparation of, 663
Humidity, effects on metabolism in tomato and apple, 217
HUNGATE, R. E., Cohesion theory of transpiration, 783
HURD-KARRER, ANNIE M., Carbohydrate and nitrogen relations in wheat plants with reference to type of growth under different environmental conditions, 533
Hydration, bound and free water in an organic colloid at different degrees of, 143
of solute ions in relation to acidity, alkalinity, and pH, 107
Hydrogen ion concentration, 453
ion concentration and germination, 522
ion concentration of culture solutions, 204
ion concentration of drainage, 426

I

Ice-formation, salt concentration and reversibility of as related to hardiness of winter wheat, 601
Illumination, effect on blind and flowering shoot production, 272
Imbibitional pressures of cell constituents and evaporation in its thermodynamic relation, 653
Incubation time, relations of germinating soy beans to length, 1
Influence of thyroxin on growth of plants, 377
Injury, losses to corn crop caused by, 749
Intensity of supplementary light, 555
International Botanical Congress, Sixth, 185, 862
Invertase studies, 458
Iodine effects, 849
Ions, hydration of solute in relation to acidity, alkalinity, and pH, 107
Iron, effect on chlorophyll production in corn, 73
humate, preparation, 663
toxicity, 441

J

Juice extraction, method for preparation of green plant material for, 839

K

KAPPERT, H., Grundriss der gärtnerischen Pflanzenzüchtung, 864

Keeping quality, relation of benzoic acid content and other constituents of cranberries, 631
Kentucky bluegrass, responses to variations in temperature, light, cutting, and fertilizing, 83
KERSTEN, H., Effect of soft x-rays on germination of wheat seeds, 173
KERTESZ, Z. I., Carbon dioxide content of the gas from pea pods, 339
Killing plant material for cryoscopic determinations, comparison of heating and freezing methods, 369
KLINKOWSKI, M., Pflanzliche Konstitutionslehre, 186

L

Laboratory, CHARLES REID BARNES Botanical, 697
Lateral water transfer in leaves of *Ginkgo biloba*, 387
LEACH, W., Respiration in plants, 862
Leaf temperatures and transpiration, 165
LEASE, E. J., Influence of shorter light rays upon absorption of nitrate by the young wheat plant, 127
Leaves of citrus trees, penetration and accumulation of petroleum spray oils, 699
of *Ginkgo biloba*, lateral water transfer in, 387
LEHMAN, BEATRICE, Nuclear division in *Tradescantia virginiana*, 845
Length of incubation time, relations of germinating soy beans to, 1
LEWIS, G. N., Tolerance of liquid-air temperatures by seeds of higher plants for sixty days, 392
Life membership award, 183
Light exposures, carbohydrate-nitrogen and base element relationships of peas grown in water culture under various, 301
intensity, elongation and expansion under reduced, 767
intensity of supplementary, 555
rays and absorption of nitrate by the young wheat plant, 127
responses of Kentucky bluegrass to variations, 83
Limiting factors, 136
LIPMAN, C. B., Tolerance of liquid-air temperatures by seeds of higher plants for sixty days, 392
Liquid-air temperatures, tolerance of by seeds of higher plants, 392
LIVINGSTON, B. E., The soil as direct source of carbon dioxide for ordinary plants, 237
LOEHWING, W. F., Physiological aspects of effect of continuous soil aeration on plant growth, 567
LONG, FRANCES L., Application of calorimetric methods to ecological research, 323
Factors in elongation and expansion under reduced light intensity, 767
Losses to the corn crop caused by leaf injury, 749
Lupinus, albus, 355
luteus, 355

M

MALLERY, T. D., Comparison of heating and freezing methods of killing plant material for cryoscopic determinations, 369
Mammalian red cells, 864
Manganese, effect on chlorophyll production in corn, 73
MARTIN, E. V., A large-capacity drying oven with constant uniform temperature and forced ventilation, 689
Effect of soil temperature on transpiration in *Helianthus annuus*, 619
Material, comparison of heating and freezing methods of killing plant for cryoscopic determinations, 369
Mechanisms involved in ascent of sap in plants, 856
Meeting, Berkeley, 695
Boston, 183
Pittsburgh, 395, 695, 861
summer, 395
MELDRUM, NORMAN U., Cellular respiration, 862
Membership award, life, 183
committee, life, 861
MERKENSCHLAGER, F., Pflanzliche Konstitutionslehre, 186

Metabolism, carbohydrate, 474
in tomato and apple, effects of humidity, 217
of etiolated seedlings as affected by ammonium nutrition, 351
protein, 471
Metals, humate, preparation, 663
Method for the preparation of green plant material for the extraction of juices, 839
Methods, calorimetric, application to ecological research, 323
chemical, 696
committee, chemical, 397
heating and freezing, of killing plant material for cryoscopic determinations, 369
Migration of ash constituents, effects of potassium upon, 399
MILLER, E. S., Absorption spectra of alpha and beta carotenes and leaf xanthophyll at room and liquid nitrogen temperatures, 179
Application of quantitative spectral analyses to binary mixtures of the common carotenoids, 681
Quantitative absorption spectra of the common carotenoids, 693
Mineral nutrients, 191
Minnesota section, 395
MITCHELL, J. W., Effects of humidity on metabolism in tomato and apple, 217
Mitosis, pathology, 864
Mycelium production of *Fusarium oxysporum*, relation of amino acids, 823

N

Narcissus, growth of, as influenced by thyroxin, 380
New England section, 184, 395, 696
NIGHTINGALE, G. T., Effects of humidity on metabolism in tomato and apple, 217
Nitrate, absorption by young wheat plants, 127
nitrogen assimilation, particularly in tomato and apple, 31
sodium, applications, effects on flower and blind wood production, 270
Nitrogen, assimilation in tomato, effects of soil pH on, 52
assimilation of nitrate, particularly in tomato and apple, 31
chemical composition of rose shoots grown with high and low, 159
differences in colloidal and non-colloidal, 275
effects of potassium upon amounts of protein and amino forms, 453
preparation of aqueous extracts of soluble, from plant tissues, 817
relations in wheat plants with reference to type of growth under different environmental conditions, 533
sources, 195
supply and effect of cutting, 93
Nitrogen-carbohydrate and base element relationships of peas grown in water culture under various light exposures, 301
ratios with respect to sexual expression of hemp, 731
NOÉ, A. C., GOTTLIEB HABERLANDT, 851
Non-aerated and aerated culture solutions, anatomical and histological differences between roots of barley grown in, 389
Nuclear division in *Tradescantia virginiana*, 845
Nutritional requirements of the root rot fungus, *Phymatotrichum omnivorum*, 187
Nutrition, ammonium, metabolism of etiolated seedlings as affected by, 351

O

Oils, cresoap emulsions of petroleum, freezing phenomena, 795
natural, in citrus, 703
petroleum spray, penetration and accumulation in leaves, twigs, and fruit of citrus trees, 699
Organic chemist, what he can do for plant physiology, 685
Osmotic pressures of cell constituents, evaporation in its thermodynamic relation, 653
Oven, a large-capacity drying, 689
OVERTON, JAMES BERTRAM, 183
Oxidase activity, effect of high-frequency sound waves, 385

P

Pahala blight, 427
Pathology of mitosis, 864
Pea pods, carbon dioxide content of the gas from, 339
Peas grown in water culture, carbohydrate-nitrogen and base element relationships of, 301
Pelagophycus porra, 145
Penetration and accumulation of petroleum spray oils in leaves, twigs, and fruit of citrus trees, 699
Peptase studies, 469
Petroleum oils, freezing phenomena in cresoap emulsions, 795
spray oils, penetration and accumulation in leaves, twigs, and fruits of citrus trees, 699
Phaseolus vulgaris, 355
pH, hydration of solute ions in relation to, 107
of soil, effects on nitrogen assimilation in tomato, 52
value of nutrient solution, 45
Phosphorus, 465
Photoperiodic response, 544
Photosynthesis, wave-length limits, 645
Phyllotaxy, fruiting apple spur, 858
Phymatotrichum omnivorum, nutritional requirements of, 187
Physiology, plant, and the organic chemist, 685
plant, recent advances in, E. C. BARTON-WRIGHT, 397
Pisum sativum, growth of in solutions containing thyroxin, 381
Pittsburgh meeting, 395, 695, 861
Plant biochemistry, W. E. TOTTINGHAM, 698
biochemistry, 863
breeding, horticultural, 864
constitution, F. MERKENSCHLAGER and M. KLINKOWSKI, 186
material, comparison of heating and freezing methods of killing for cryoscopic determinations, 369
physiology, and the organic chemist, 685
physiology, recent advances in, E. C. BARTON-WRIGHT, 397
tissues, preparation of aqueous extracts of soluble nitrogen, 817
Plants, influence of thyroxin on growth, 377
soil as direct source of carbon dioxide for ordinary, 237
thermodynamics of, KURT STERN, 397
tolerance of liquid-air temperatures by seeds of higher, 392
PLATENIUS, HANS, Chemical changes in carrots during growths, 671
Poa pratensis, 84
Pods, pea, carbon dioxide content of gas from, 339
POLLITZER, GEORG, Pathologie der Mitose, 864
PONDER, ERIC, The mammalian red cells and the properties of haemolytic systems, 864
Potassium, effects upon amounts of protein and amino forms of nitrogen sugars, and enzyme activity of sugar cane, 453
effects upon growth of sugar cane and absorption and migration of ash constituents, 399
rôle of, 482
Preparation, of aqueous extracts of soluble nitrogen from plant tissues, 817
of green plant material for the extraction of juices, 839
Pressures, imbibitional and osmotic, of cell constituents, evaporation in its thermodynamic relation, 653
Prize committee, STEPHEN HALES, 397
Production, chlorophyll, under various environmental conditions, 59
of alcohol and acetaldehyde by tomatoes, 359
Program committee, 396
Progress of botany, FRITZ VON WETTSTEIN, 398
Protein, effects of potassium upon amounts, 453
metabolism, 471
Pruning experiments, 269
Publication, regulations, 184
Purdue section, 396

Q

Quantitative, absorption spectra of the common carotenoids, 693
spectral analyses, application to binary mixtures of the common carotenoids, 681
Quinic acid, 634

R

Radishes, 60
Ratios, carbohydrate-nitrogen, with respect to sexual expression of hemp, 731
Rays, influence of shorter light, upon absorption of nitrate by the young wheat plant, 127
Recent advances in plant physiology, E. C. BARTON-WRIGHT, 397
Red cells, mammalian, 864
spruce, germination, 491
Regulations of publication, 184
Requirements, nutritional, of the root rot fungus, 187
Research, chlorophyll and carotenoids, 697
ecological, application of calorimetric methods to, 323
Researches on fungi, A. H. R. BULLER, 398
Respiration, 862
root, and absorption, relation between, 283
Respiratory rate and enzyme activity as related to the hardened condition of plants, 831
Responses of Kentucky bluegrass to variations in temperature, light, cutting, and fertilizing, 83
Reversibility of ice-formation as related to hardiness of winter wheat, 601
Ripening experiments, 520
ROHRBAUGH, P. W., Penetration and accumulation of petroleum spray oils in leaves, twigs, and fruit of citrus trees, 699
Root respiration and absorption, relation between, 283
rot, fungus, *Phymatotrichum omnivorum*, nutritional requirements of, 187
tips, growth of excised tomato in liquid medium, 585
Roots of barley grown in aerated and non-aerated culture solutions, 389
Rosa multiflora, 157
Rose shoots, gradient composition of, 157
Roses, causes of blind wood in, 261
RUNYON, E. H., Demonstration of mechanisms involved in ascent of sap in plants, 856

S

Salt concentration of ice-formation as related to hardiness of winter wheat, 601
SAMISCH, RUDOLF, Effect of high frequency sound waves on oxidase activity, 385
Sap ascent in plants, mechanisms involved, 856
physico-chemical studies of expressed, 453
Sclerotia production, synthetic media as influencing, 212
Section, New England, 184, 395, 696
Minnesota, 395
Purdue, 396
Seedlings, etiolated, metabolism of as affected by ammonium nutrition, 351
Seeds, tolerance of liquid-air temperatures by, 392
wheat, effect of soft x-rays on germination, 173
Sexual expression of hemp and carbohydrate-nitrogen ratios, 731
SHIVE, J. W., Preparation of aqueous extracts of soluble nitrogen from plant tissues, 817
Shoots, rose, gradient composition of, 157
SHULL, C. A., Evaporation in its thermodynamic relation to the cohesion theory and to imbibitional and osmotic pressures of cell constituents, 653
Lateral water transfer in leaves of *Ginkgo biloba*, 387
Sixth International Botanical Congress, 185, 862
SKINNER, C. E., Production of vitamins

by a pure culture of *Chlorococcum* grown in darkness on a synthetic medium, 807
Sodium: can it replace potassium, 443
nitrate applications, effects on flower and blind wood production, 270
Soil, as direct source of carbon dioxide for ordinary plants, 237
pH, effects on assimilation of ammonium and nitrate nitrogen in tomato, 52
reaction, 556
temperature, effect on transpiration in *Helianthus annuus*, 619
Solids in watermelon, soluble, 181
Soluble solids in watermelon, 181
Solute ions in relation to acidity, alkalinity, and pH, hydration of, 107
Solutes, transpiration and distribution of inorganic, 168
Solutions, barley roots grown in aerated and non-aerated culture, 389
Sound waves, effect of high frequency on oxidase activity, 385
Source of carbon dioxide for ordinary plants, the soil as direct, 237
Soy beans, 60, 574
beans, germinating, relations to temperature and length of incubation time, 1
Spectra, absorption, of alpha and beta carotenes, 179
quantitative absorption of common carotenoids, 693
Spectral analyses, application of quantitative, to binary mixtures of the common carotenoids, 681
Spray oils, petroleum, penetration and accumulation in leaves, twigs, and fruit of citrus trees, 699
Spruce, red, germination, 491
Staling products, preliminary test for, 205
STEELE, CATHERINE CASSELS, An introduction to plant biochemistry, 863
STEPHEN HALES prize committee, 397
STEPHENS, H. L., Influence of shorter light rays upon absorption of nitrate by the young wheat plant, 127
STERN, KURT, Thermodynamics of plants, 397
STILES, W., Respiration in plants, 862
Storage, influence on carbon dioxide content of pea pods, 846
STREET, O. E., Carbohydrate-nitrogen and base element relationships of peas grown in water culture under various light exposures, 301
Sugar cane, effects of potassium, 399
cane, effects of potassium upon amounts of protein and amino forms of nitrogen, sugars, and enzyme activity, 453
Sugars, effects of potassium upon amounts of protein and amino forms, 453
Summer meeting, 395
Sunflowers, 569

T

TALLEY, P. J., Carbohydrate-nitrogen ratios with respect to sexual expression of hemp, 731
TAUBENHAUS, J. J., Nutritional requirements of the root rot fungus, *Phymatotrichum omnivorum*, 187
Temperature, a drying oven with constant uniform, 689
effects, 510, 556
effects on nuclear division, 845
of soil, effect on transpiration in *Helianthus annuus*, 619
relations of germinating soy beans, 1
responses of Kentucky bluegrass to variations in, 83
transpiration as a buffer, 167
Temperatures, absorption spectra of alpha and beta carotenes and leaf xanthophyll at room and liquid nitrogen, 179
leaf, and transpiration, 165
tolerance of liquid-air by seeds of higher plants, 392
Theory, cohesion, of transpiration, 783
Thermodynamic relation of evaporation to the cohesion theory, 653
Thermodynamics of plants, KURT STERN, 397
Thyroxin, influence on growth of plants, 377
TIEDJENS, V. A., Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in apple and tomato, 31
Time, length of incubation, relations of germinating soy beans, 1

Tip to base, gradient composition of rose shoots from, 157
Tolerance of liquid-air temperatures by seeds of higher plants for sixty days, 392
Tomato, assimilation of ammonium and nitrate nitrogen, 31
effects of humidity on metabolism, 217
root tips, growth of excised in liquid medium, 585
Tomatoes, production of alcohol and acetaldehyde by, 359
TOTTINGHAM, W. E., Influence of shorter light rays upon absorption of nitrate by the young wheat plant, 127
Toxicity, iron, 441
Tradescantia virginiana, nuclear division, 845
Transfer, lateral water, in leaves of *Ginkgo biloba*, 387
Transpiration, cohesion theory, 783
significance of, 165
TUCKER, L. R., Soluble solids in watermelon, 181
TUKEY, H. B., Gradient composition of rose shoots from tip to base, 157
TURNER, DOROTHY M., British economic grasses, 186
Twigs of citrus trees, penetration and accumulation of petroleum spray oils, 699

U

Ultra-violet light, effect on carbohydrate content of stored apples, 637
ULVIN, G. B., Chlorophyll production under various environmental conditions, 59

V

Velocity of solute ions in relation to acidity, alkalinity, and pH, 117
Ventilation, a drying oven with forced, 689
Vicia faba, growth of as influenced by thyroxin, 380
VICKERY, H. B., What the organic chemist can do for plant physiology, 685
Vitamin production by a pure culture of *Chlorococcum* grown in darkness on a synthetic medium, 807

W

Water, bound and free, in an organic colloid at different degrees of hydration, 143
culture, carbohydrate-nitrogen and base element relationships of peas, 301
melon, soluble solids in, 181
transfer, lateral, in leaves of *Ginkgo biloba*, 387
Wave-length limits of photosynthesis, 645
Waves, effect of high frequency sound on oxidase activity, 385
WETTSTEIN, FRITZ VON, Progress of botany, 398
Wheat, carbohydrate and nitrogen relations, 533
influence of shorter light rays upon absorption of nitrate by, 127
salt concentration and reversibility of ice-formation as related to winter hardiness, 601
seeds, effect of soft x-rays on germination, 173
WHITE, P. R., Potentially unlimited growth of excised tomato root tips in a liquid medium, 585
WILLCOX, O. W., Reshaping agriculture, 863
Wood, blind, in roses, causes of, 261

X

Xanthophyll, absorption spectra of leaf, 179
X-rays, soft, effect on germination of wheat seeds, 173

Y

YOUNG, P. A., Freezing phenomena in cresoap emulsions of petroleum oils, 795
YOUNG, T. F., Evaporation in its thermodynamic relation to the cohesion theory and to imbibitional and osmotic pressures of cell constituents, 653

Z

Zea mays saccharata, 73
ZECHMEISTER, L., Carotinoids, 862

I. A. R. I. 75.

IMPERIAL AGRICULTURAL RESEARCH
INSTITUTE LIBRARY
NEW DELHI.

Date of issue.	Date of issue.	Date of issue.
19-12-38		
1-3-41		
5-4-48		
16-4-57		
27-10-59		
5-12-55		
30-8-57		
18-1-65		
17-10-68		